Proceedings of the Third 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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Aquaculture Feed Grains Program - Situation Analysis

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Introduction

Since 2003 the Grains R&D Corporation has invested 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, 2003), in May 2003, it was supported that the project should proceed to an expanded second phase.

In the second phase of the project additional investment has been obtained 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 has improved 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. It was agreed that under the expanded format, with multiple component projects, that the project should be recognised as a program.

Lupin Production and Market Trends

Annual lupin production over the past five years (1999 – 2004) has averaged at 1.24 million tonnes per annum (Wilkins and Robertson, 2005). The EU is the next biggest producer with an annual crop of around 62,000 tonnes per annum. Lupin production is predominant in the Mid-west region of WA where soil and climatic characteristics for the crop are favourable. However, lupin production in Australia and particularly in Western Australia (WA) has been declining in recent years. This is a response to many variables including a strengthening sheep/wool sector, drought and comparatively low returns from cropping lupins. While other land use and drought factors are difficult to influence, improving profitability for lupins is being pursued on several fronts.

Grain prices in recent years have been particularly variable as a result of both drought and currency value fluctuations (Table 1). Despite the comparatively high prices paid for the grain, low yields make lupins an expensive crop to produce. Substantial effort is being spent on improving crop yields, as well as improving grain characteristics.

Table 1. Prices paid in WA for key grain varieties 2000 – 2005.

Grain	1999-2000	2000-01	2001-02	2002-03	2003-04	2004-05
Lupin kernel meal	281	357	393	431	364	293
Lupins	162	215	240	267	220	170
Canola	307	342	400	500	425	360
Feed Barley	163	185	203	242	175	157
Malting Barley	203	230	248	307	195	192
Feed Wheat	156	180	172	230	202	150
Wheat (APH)	234	262	285	290	248	216

Data from Countryman (2001 – 2005). Prices are A$, GST exclusive. Lupin kernel meal price based on 70% yield + $50/tonne.

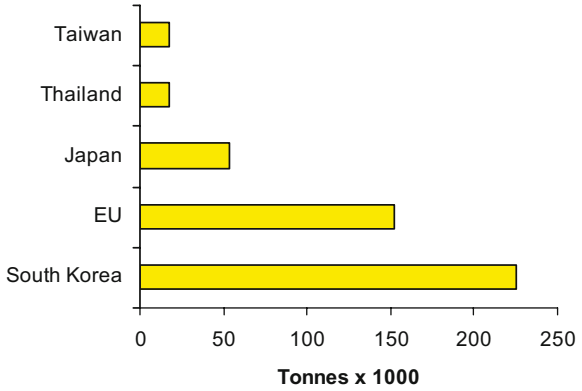

Figure 1. Exports of lupins from Western Australia. Data are three year annual averages (2000 – 2003). Data from ABS and Department of Agriculture (Wilkins and Robertson, 2005).

Domestic lupin use in WA is limited and there is limited lupin trade between Eastern and Western Australia. Traditionally the majority of lupin production is exported, although in drought years substantial tonnage is kept on-farm as a livestock feed. Key export markets for lupins include East Asia (Korea and Japan) and the EU (Spain and Netherlands). There is some trade to South East Asia (Figure 1) (Wilkins and Robertson, 2005).

Nationally, lupins are the fourth largest export crop after wheat, barley and canola. About 75% of that lupin export originates from WA. Western Australia also dominates national exports of wheat, canola and oats.

Global production of lupins, at around 1.5 million tonnes, is tiny compared to annual soybean meal production of around 125 million tonnes (Figure 2). Based on this, the prospect for the lupin kernel meal market 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. 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.

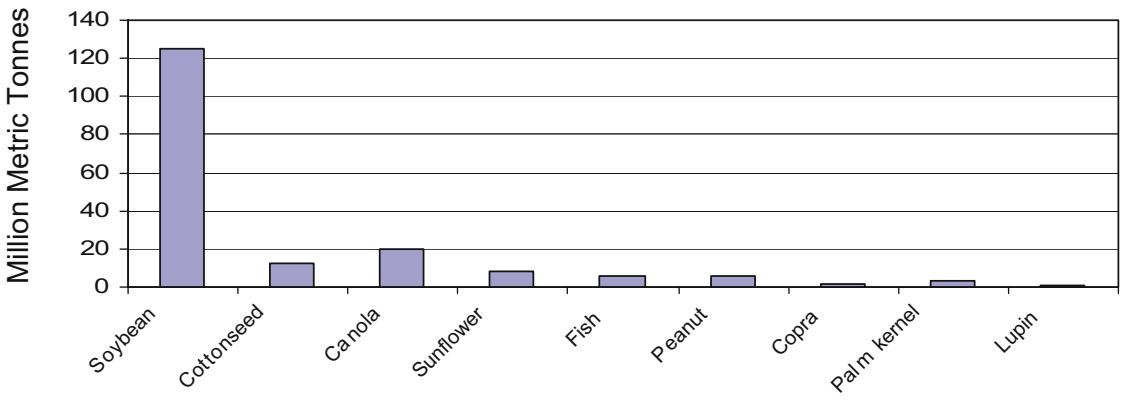

Figure 2. Global production of key plant protein meals in 2001/2002.

Which Aquaculture 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 2).

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.

Table 2. Global 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

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 3). 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 3. 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 (g/kg)	250	300	450	450
Diet Fat (g/kg)	70	80	90	250
Fish meal ($1200)	9.0	11.0	33.0	42.2
Crustacean Meal ($1500)	0.0	0.0	10.0	0.0
Fish oil ($1000)	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.

Aquaculture Market Progress

Specific market intelligence about the amount of feed grains used by the aquaculture feeds sector has been difficult to obtain. Largely it is based on feedback direct from the industry about the volumes they use, though we have been unable to cross-reference this with data from other sources.

Despite these data limitations, crude estimates of the volume of lupins used by several major companies in Australia and Worldwide have been obtained. The largest volume used in the aquaculture feed sector has been in the Atlantic salmon industry. In 2004 about 15,000 tonnes was used in Norway, with about an additional 15,000 tonnes used in Chile. The Norwegians have used Australian (*L. angustifolius*) grain (sourced via Netherlands), while the Chileans have used locally produced grain (*L. albus*). Other significant markets include Australia (~5,000 tonnes), UK (~1,000 tonnes) and Japan (~1,000 tonnes). All of the grain for these markets has been *L. angustifolius* of Australian origin.

In 2005 it is expected that we will see a significant reduction in the total volume of lupins used on aquaculture feed rations globally. This will be driven by two key factors; the strong Australian dollar making international trade in Australian grain comparatively expensive and a massive 2004 international soybean crop, which has resulted in the lowest soybean meal prices in many years. This has placed downward pressure on lupin prices that may further influence lupin production.

Revised Project Objectives

The objectives of the new program were altered from the original project 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, progress and the future 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.

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Implications of variability amongst Lupin cultivars in processing

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Introduction

The nutrient content of lupins in terms of protein, amino acids, energy, minerals etc. has been established and widely accepted by stockfeed manufacturers. However, there are significant differences between cultivars that are less widely known by the stockfeed industries. In recent times, differences observed among lupin varieties have raised questions in terms of nutritional variability among lupin varieties. Notably Hughes et al. (1998) reported apparent metabolizable energy (AME) values of *L.angustifolius* cv Gungurru fed to broiler chickens ranging from 6.53 to 11.00 MJ $kgDM^{-1}$. Glencross et al. (2003) also noted variability in fish performance; with fish fed the Gungurru lupin based diet growing significantly slower than those on the Warrah lupin based diet. According to van Barneveld et al (1994), 2 MJ/Kg energy could be worth up to $30/tonne. In this paper we have take the opportunity to attempt a retro-analysis, considering the possible causes for these differing nutritive values. Modelling within the known natural variations of macronutrient composition and their possible effect on animal response (See Table 6). Hughes et al. (1998) reported the AME values ranging from 9.8 to 12.3 MJ/kg for the cv Gungurru, grown at 3 different Western Australian sites in the 1994/95 harvest (Table 4), using 30% inclusion of lupin kernel meals in a sorghum/casein based diet.

Table 4. Effects of site on protein, fat and AME of *L. angustifolius* cv Gungurru.

Location	Balla	Walkaway	Dongara
*Kernel %CP 'as is'	40.6	36.8	35.5
Ileal viscosity cP	3.4	8.9	3.5
AME (MJ/kg)	12.3	9.8	10.2
Whole seed 'as is'		g/100 g	
Fat	5.1	5.6	5.7
Moisture	9.5	9.5	9.5
CP (N*6.25)	31.1	28.2	285

* kernel and hull were separated with virtually negligible losses

The crude protein (CP), of the original whole seed as well as the CP of the kernel meals was analysed. This allowed us to estimate relatively accurately the dehulling efficiency needed to effect the increase in protein concentration achieved. However we needed to make some assumptions as to the levels of other constituents that were not measured.

The composition of a 'typical lupin seed' is described in Fig 3. It is quite apparent the kernels are made up of 4 major constituents, Protein (40%), Cell Wall Material (30%-Kernel Non Starch Polysaccharides-NSP), Fat (7%), and Oligosaccharides (6%). When one of these constituents goes up then another must diminish correspondingly, and it is this particular interchange that was considered in our efforts to understand the differing energy values reported.

Natural variations : Protein

Figure 4, shows the range of protein values for whole seed *L. angustifolius* 'as is' in the GRAILE database as of 1994, highlighting the large range of protein values possible.

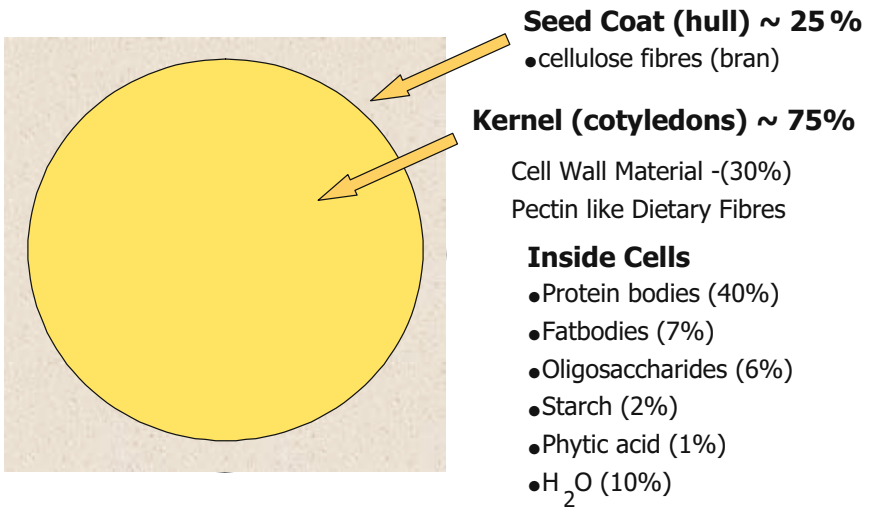

Figure 3. Composition of Narrow leafed lupin (NLL).

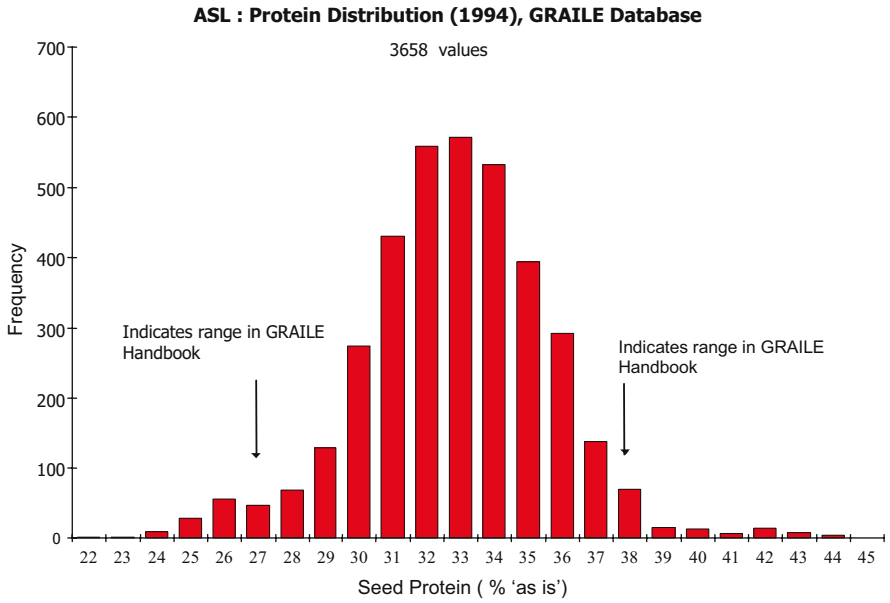

Figure 4. Protein values for Narrow leafed lupin (NLL) from the GRAILE database.

Nitrogen – Not always Protein

Crude Protein is measured as **Nitrogen x 6.25,** assuming all nitrogen is protein. Blagrove et al (1976) highlighted the non-protein nitrogen (NPN) fraction in lupins, and how it varied depending on environment and cultivar (Table 5). Three *L. angustifolius* cultivars grown under varying soil sulphur concentrations, showed no difference in the total nitrogen content of the seed, in two cultivars, but the NPN fraction increased with low soil sulphur in all cases.

Table 5. Effect of soil sulphur on nitrogen, and non-protein nitrogen in the lupin seed.

L. angustifolius (dry - defatted whole meal)						
	Uniharvest		CPI 47644		Fest	
Soil Sulfur (ppm)	High	Low	High	Low	High	Low
%N in seed	5.4	5.4	4.9	5.1	6.2	5.3
Non protein N	0.7	0.9	0.8	1.7	0.3	0.4
%N as protein in seed	4.7	4.5	4.1	3.4	5.9	4.9
Cys+Meth (g/16gN)	2.1	0.6	2.4	0.9		
%CP in-defat meal	33.8	33.8	30.6	31.9	38.8	33.1
%N as protein	29.4	28.1	25.6	21.3	36.8	30.9
%CP Whole seed 'as is'	28.9	28.9	26.2	27.3	33.1	28.3
True protein	**25.1**	**24.0**	**21.9**	**18.2**	**31.5**	**26.4**

Other macronutrients

Evans (1994), reported values for starch and sucrose in Gungurru kernels: Starch: 0.6% db (0.54% as is); therefore in whole seed ~ 0.4 % as is Sucrose: 3.8% db (3.4% as is); therefore in whole seed ~ 2.5% as is Cell Wall Material (Dietary fibre): 29% db (26% as is); in whole seed ~20% as is.

From the GRAILE handbook, Petterson et al. (1977). Data are %.

	mean	range	Sample no
Ash	2.7	2.2 –3.2	180
lignin	0.8	0.4 –1.9	111
Phytate/tannins/alkaloids	0.8	0.5 – 1.4	236
Oligosaccharides	4.0	2.9 – 5.2	202

Implications of NSP

Hughes et al. (1998) reported the effect of lupin kernel NSP (cell wall material) on AME in a dose response experiment. Refined, isolated lupin kernel fibre was included in a sorghum/casein based diet at inclusion levels of 0%, 5%, 10% & 15%, chosen to represent inclusion rates of 0% 10%, 20% & 30% whole seed Gungurru. Lupin NSP significantly depressed the AME of the diet; for every 1% NSP in the diet the AME was lowered by 0.288MJ (Fig 5).

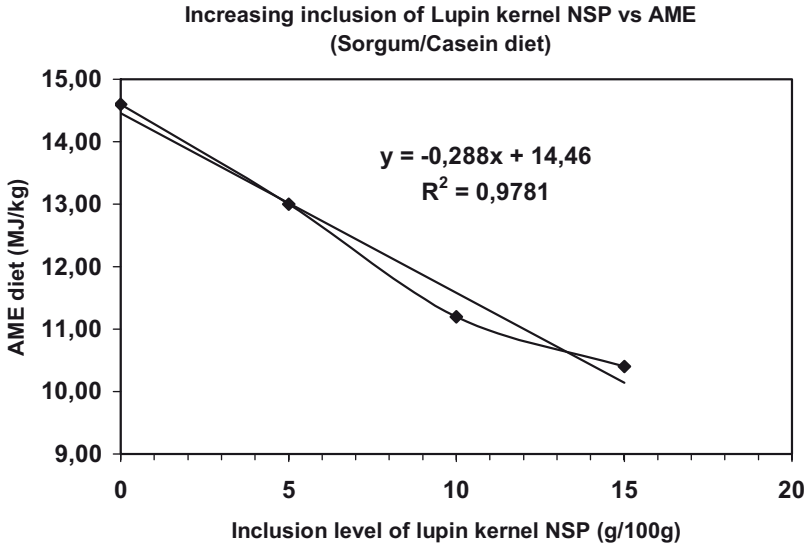

Figure 5. Effect of Lupin kernel NSP on the AME of a sorghum/casein base diet in broiler chickens.

Composition is the Key

Table 6 shows the three hypothetical Gungurru whole seed samples within a model. Fat, moisture and protein are actual measurements. Seed coat, starch, ash, lignin and phytate/tannin/alkaloids are estimates and are kept the same across the three samples for simplicity. Sucrose and oligosaccharides are varied around the average. Kernel NSP was allowed to be the model variable to complete the 100% composition. The next step was dehulling which gave kernel meals with protein concentrations close to the measured values in Table 4.

The Energy column was estimated using the energy values of the three macronutrients Fat = 39MJ/kg, Protein = 23MJ/kg and Carbohydrates = 17MJ/kg. At this point a 'what if' the NPN is 0.2% for the Balla sample and 0.7% for Walkaway and Dongara samples, was incorporated which related to the ***True Protein***. As 5% of the fat in narrow leaf lupins are waxes (and presumably indigestible) only 95% (❖***Fat minus wax***) of the fat was considered as providing energy. The energy contribution by Oligosaccharides was estimated to be 0.8MJ/kg DM when lupin kernel was included at 30% in a sorghum/casein basal diet (Hughes et al 1998). Therefore an amount of 2.5 MJ/kg was attributed to the sample with the most oligosaccharides (4.2%) and then the other samples were given a ratio i.e. Dongarra [(4.0 *2.5)/4.2 =2.4Mj/kg.].

The NSP contribution ***(❊30% NSP; -0,288X)*** of a 30% inclusion rate was estimated and then the AME depression factor of -0.2888X was utilised.

Table 6. Hypothetical composition of cv Gungurru (3 sites), influence of macronutrients and their contribution to energy in a broiler diet.

L. angustifolius Gungurru 1994/95 harvest	**Balla**			**Walkalway**			**Dongarra**		
	whole seed	kernel	Energy	whole seed	kernel	Energy	whole seed	kernel	Energy
	g/100g	g/100g	MJ/Kg	g/100g	g/100g	MJ/Kg	g/100g	g/100g	MJ/Kg
Seed Coat	22.9	2.8		22.9	1.8		22.9	5.0	
Moisture	9.5	9.7		9.5	9.7		9.5	9.8	
Protein	31.1	40.2		28.2	36.9		28.5	35.9	
****True Protein***	*29.9*	*38.6*	*8.9*	*23.8*	31.1	*7.2*	*24.2*	30.4	*7.0*
Fat	5.1	6.6		5.6	7.3		5.7	7.2	
❖Fat minus wax		*6.3*	*2.4*		*7.0*	*2.7*		*6.8*	*2.7*
starch	0.4	0.5	0.1	0.4	0.5	0.1	0.4	0.5	0.1
Ash	2.7	3.5		2.7	3.5		2.7	3.4	
lignin	0.8	1.0		0.8	1.0		0.8	1.0	
Phytate/tannins/alkaloids	0.8	1.0		0.8	1.0		0.8	1.0	
Kernel Fibre (NSP)	19.9	25.7		23.5	30.8		22.3	28.1	
****30% NSP; -0,288X***		*7.7*	*-2.2*		*9.2*	*-2.7*		*8.4*	*-2.4*
Oligossacharides	4.2	5.4	2.5	3.3	4.3	2.0	4.0	5.0	2.4
sucrose	2.6	3.4	0.6	2.3	3.0	0.5	2.4	3.0	0.5
Total sum	100.0	100.0		100.0	100.0		100.0	100.0	
Modelled AME			**12.3**			**9.8**			**10.2**

Summary

The closeness of the calculated and the measured values was a surprising result. It would seem it is possible to predict the energy value of a sample in a feeding system if enough is known of its composition and the implications of each constituent in that system. It is prudent that we truly understand the "true variations" in our grain and the "true implications" to diet formulators and processors.

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Can we manipulate lupin grain protein by farming practices?

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Introduction

The key messages of this presentation are:

1. The only practical option for growers is to choose an appropriate cultivar.
2. Processors might be able to exploit the fact that, on average, lupins grown in low rainfall areas have higher grain protein than in high rainfall areas.
3. There is potential to add value by grading out small, low protein seed.

Options for growers

GRDC funded a joint project between the Department of Agriculture and Pierre Fiévez and Associates to investigate whether lupin grain protein could be raised through crop management. An extensive series of field trials showed that the only factor to consistently affect grain protein without causing an unacceptable yield penalty was cultivar. Other options studied were N fertiliser application, either at seeding or during reproductive growth; S, P, K and trace element nutrition, herbicide and weed management, sowing rate and row spacing. All of these factors either had no effect on grain protein, or very small, inconsistent, effects. Delaying sowing usually results in higher protein, but this is associated with large yield losses.

Table 7 lists the relative protein and yield levels of current lupin cultivars. Unfortunately, some of the most recently released high yielding cultivars are at the low end of the protein spectrum, and there are some theoretical reasons why yield and protein might be negatively correlated. However, current lupin cultivars are a long way from the yield ceiling (we hope!) and there is no reason why both yield and protein cannot be further improved together. Now that lupin breeders are giving more weight to grain protein we expect this to happen and the line WALAN 2173 is the first example of this.

Table 7. Protein content and relative yield of lupin cultivars. Data taken from the Department of Agriculture's Crop Variety Testing trials since 1988.

Cultivar	Relative yield	Protein (%)
Danja	90	31.6
Yandee	93	31.7
Tallerack	95	33.2
Myallie	97	33.4
Merrit	100	32.4
Kalya	105	31.6
Tanjil	107	31.4
WALAN 2173	108	34.1
Belara	114	30.2
Mandelup	116	31.0
Quilinock	117	32.0

At the moment the price paid for high protein grain will help determine whether a variety like WALAN 2173 is grown in preference to Mandelup. On the basis of the figures in Table 7 you would have to pay 7% more for WALAN 2173 to make it equally attractive to the high yielding Mandelup. At a base price of $200/tonne, this an extra $14/tonne to get the extra 3% protein, or $4.67/% protein.

Exploiting environmental variation in grain protein

An option for processors seeking high protein lupins is to exploit the considerable environmentally induced variation in grain protein in sourcing their grain. An analysis of the Department of Agriculture's Crop Variety Testing data over an 11 year period by Cowling and Tarr (2004) showed that variation between locations and years was 6 times as great as that between cultivars. They suggest that there is no consistency in the relative protein levels produced at individual locations from year to year. However, the way they defined locations hid broad regional differences that are apparent in our own analysis of the CVT data, and from our other work.

The critical points to note from this are that protein tends to be highest in low rainfall zones, and lowest in high rainfall coastal zones. The best way to exploit this information is not clear since Cowling and Tarr's analysis suggests that developing a long term relationship with one grower, or a few growers in a relatively restricted geographical area may not lead to consistently high protein levels every year, and this issue needs further exploration.

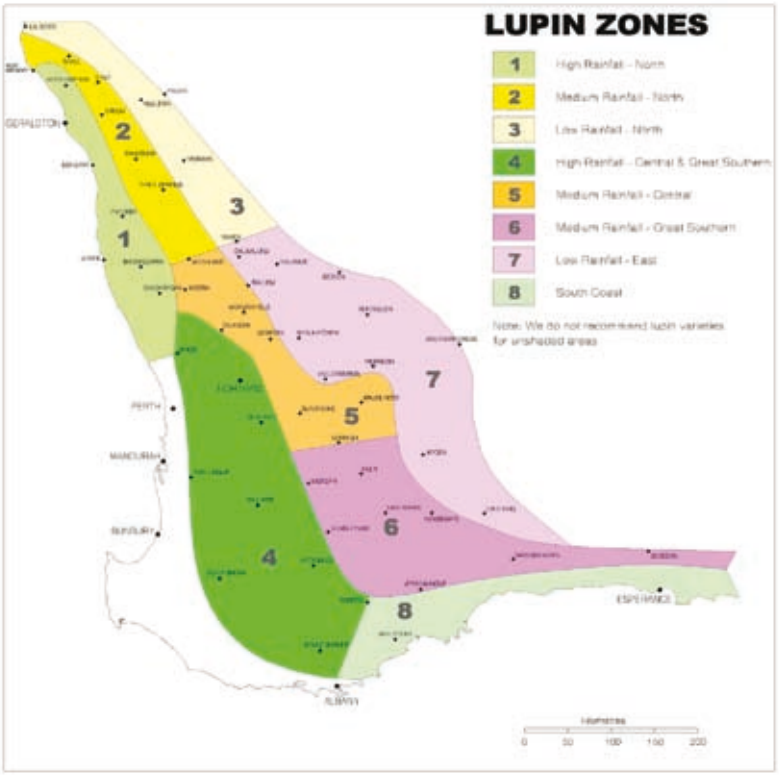

Figure 6. Map of Western Australia's Agricultural areas showing the location of lupin agzones.

Table 8. Protein content produced in Department of Agriculture's Crop Variety Testing trials in different lupin producing areas of Western Australia.

Agzone	Protein (%)
1	33.6
2	34.9
3	**35.9**
4	34.6
5	34.5
6	34.6
7	**35.9**
8	33.5

Can grading lupins on the basis of size improve protein content?

The bulk of the protein in lupin seeds resides in the kernel rather than the seed coat. Because the seed coat makes up a larger proportion of small than of large seeds, large seeds tend to have higher protein. Mark Sweetingham showed several years ago that, in samples grown at Wongan Hills, the largest 20-30% of seed had, on average, 1.25% higher protein than the smallest 10-20%, and this was consistent across cultivars (except for Merrit).

Table 9. Increasing protein content of lupin seed by grading on the basis of size. Seed from Department of Agriculture variety trail at Wongan Hills 1999.

Cultivar	Small fraction		Large fraction	
	% in this fraction	Protein (%)	% in this fraction	Protein (%)
Belara	15	33.4	21	34.8
Tanjil	14	35.4	19	36.9
Merrit	10	37.3	27	37.3
Tallerack	18	36.0	22	38.1
Myallie	7	37.5	50	38.6

I looked at the seed size distribution of lupins from a trial at Merredin in 2002 in more detail (Figure 7).

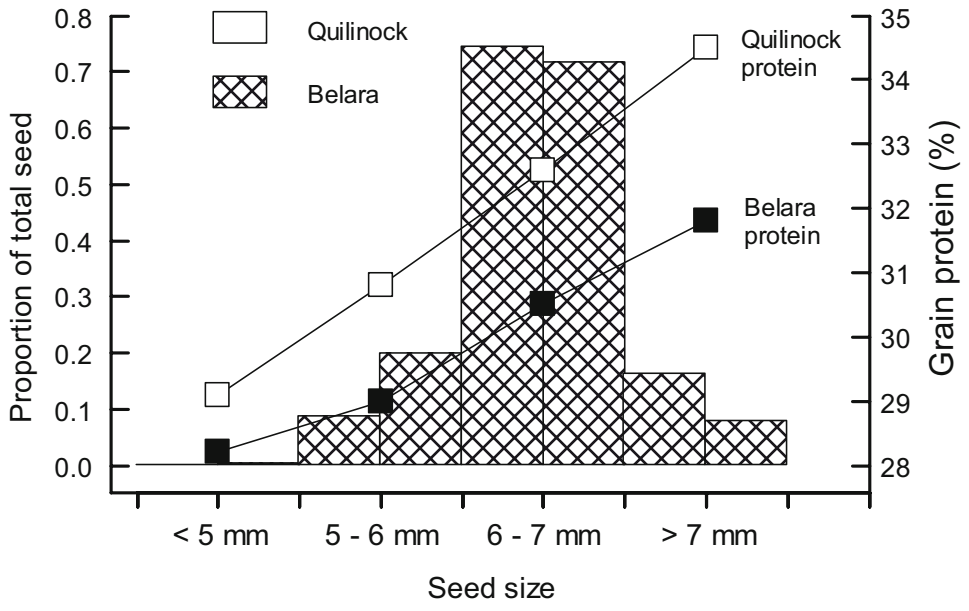

Figure 7. Seed size distribution of lupins grown at Merredin in 2002, and grain protein of the different seed size fractions.

Seed retained on a 7 mm sieve, which made up 16% of the seed in Quilinock and 8% in Belara, had 3-4% higher protein than seed in the 5-6 mm fraction. From these data, I estimate that seed larger than 6.5 mm would, in the case of Quilinock, make up 50% of the total seed lot and have an average protein of 33.4%, compared to 32.7% of the ungraded sample. Whether grading is worthwhile economically, and the optimum size cut-off, will depend on the costs of the operation and on the value of increased protein to the particular end-use.

References

W.A. Cowling and A. Tarr (2004). Effect of genotype and environment on seed quality in sweet narrow-leafed lupin (*Lupinus angustifolius* L.). *Australian Journal of Agricultural Research* **55,** 745-751.

Extracting value from protein variation in lupins

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Key Messages

Protein content in lupins varies across species, varieties, years and locations. In theory the nature and extent of the variation, and its information asymmetries, provide commercial opportunities and advantages. In practice, however, the additional returns generated by exploiting protein differentials may be limited due to the small premia paid for additional protein and the logistical, handling and marketing costs associated with forming and utilising segregations. From the farmers' perspective their main gains remain in yield and unit cost of production reduction rather than protein enhancement and the same, to some degree, may apply to the entire industry.

Aims

To provide economic information about the nature and extent of variation in lupin protein.

Method

Apply economic concepts and models to examine the value contained in protein variation in lupins.

Data

Variation in the protein of lupins is known to depend on species, variety, year, location and to a limited extent, crop management. Figure 8 is an illustration of variation across varieties whilst Table 10 lists differences between species in protein content.

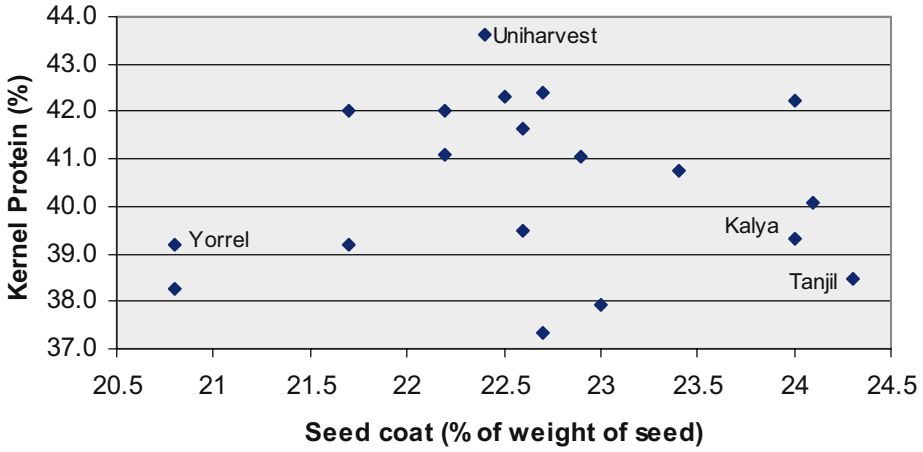

Figure 8. Variation in lupin content across varieties.

Table 10. Major components of whole grains of various lupin species (%).

Species	*L. angustifolius*		*L. albus*		*L. luteus*		*L. mutabilis*	
	Seed	Kernel	Seed	Kernel	Seed	Kernel	Seed	Kernel
Seed Coat	23	0	18	0	27	0	16	0
Moisture	9	12	9	11	9	12	9	10
Protein	32	41	36	44	38	52	44	52
Fat	6	7	9	11	5	7	14	17
Ash and Lignin	4	4	4	5	4	5	5	5
Polysaccharides	22	28	17	21	8	10	9	10
Oligosaccharides	4	6	7	8	9	12	?	?

Variation in protein (and oil) content is also known to occur within production of each variety and is related to seed size as shown in Table 11. By grading seed of a particular variety (or even sets of varieties) it is possible to separate out larger seed sizes that have higher protein levels. However, in most cases such grading is likely to be uneconomic. The reasons are a combination of the market place not offering for whole seed a sufficiently high premium for additional protein. Often this premium is less than $4 per percent above some threshold. The increase in protein content achieved through grading is not high, typically no more than 2 percent. Lastly, proportion of grain that is segregated into a larger seed and higher protein category is rarely more than 30 percent.

Table 11. Variation in protein and oil content according to seed size.

Variety	Small seed fraction				
	Proportion of all seed of this variety that is classed as small seed %	Mean seed weight (mg)	Seed coat %	Protein %	Oil %
Belara	15	75	31.1	33.4	5.4
Tanjil	14	76	29.7	35.4	5.3
Merrit	10	76	30.8	37.3	5.4
Tallerack	18	80	28.8	36.0	5.3
Myallie	7	76	29.3	37.5	5.0
Average	-	-	29.9	35.9	5.3
Variety	**Large seed fraction**				
	Proportion of all seed of this variety that is classed as large seed %	Mean seed weight (mg)	Seed coat %	Protein %	Oil %
Belara	21	146	24.6	34.8	7.1
Tanjil	19	143	25.0	36.9	6.7
Merrit	27	142	23.8	37.3	6.6
Tallerack	22	153	23.4	38.1	6.3
Myallie	50	168	22.6	38.6	6.3
Average	-	-	21.5	37.1	6.6

Source: Department of Agriculture variety trials at Wongan Hills Research Station in 1999

As an illustration of the merits of grading for seed size:

Cost of whole seed	$/t	185
Cost of grading	$/t	20
Proportion classed as large seed	%	25
Additional protein in large seed	%	2
Premium for extra protein	$/%	4
Revenue from grading	$/t	187
Net revenue from grading	**$/t**	**167**

In this case the rational decision would be not to grade. However, each season there is a natural grading that occurs by virtue of the location and varieties grown. In this case the grain marketer has no grading costs to incur other than acquisition of knowledge of the spatial pattern of grain protein by location or stack. In that setting additional value can be derived by exploiting this knowledge of protein variation. How much value is conditional on the logistical costs of stack management and market premia for whole grain lupin content.

For those who wish to extract value from de-hulling then there are definite advantages in purchasing certain varieties, particularly those with larger seed (and or smaller seed coats) and reasonably high whole seed protein levels. The advantage of the larger seeds (or smaller seed coats) is that the kernel yield is higher and the margins associated with dehulling are greater.

Conclusion

There are variations in the protein content of lupins across varieties, species, locations and years. Such variation may or may not afford economic opportunities in the handling and processing of lupins. The existence, nature and size of these economic opportunities depend on several physical, cost and price relationships.

Adapting lupins to use in fish diets - Benefits and Constraints

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Introduction

Like all feed ingredients, lupin products also have some limitations to their use in aquaculture feeds. In this paper, these limitations are examined on a range of issues, including compositional variability, nutritional variability, anti-nutritional factors and palatability limitations. Progress in evaluation of lupin protein concentrates is also examined. Limitations to functional properties when included in extruded salmonid feeds are covered in additional papers within the proceedings.

Compositional and Nutritional variability

In 2004 the program went through a redevelopment of the objectives, based on 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 had shown that there was substantial variability in nutritional value among the different *L. angustifolius* cultivars (Glencross et al., 2003).

In 2004 a set of 15 cultivars of *L. angustifolius* was obtained from the Wongan Hills Research Station. These 15 whole-seed samples were dehulled and kernel meals prepared from each for evaluation of their digestible nutrient and energy value. The 15 varieties proved to be unusual in that they all had atypically high levels (Figure 9). Initially it was thought that this may have been due to analytical error and repeats and external testing was undertaken to confirm this anomaly. In late 2004 each of the 15 cultivar samples, plus additional reference ingredients (including further lupin meals) were evaluated for their digestible nutrient and energy value (Figure 9).

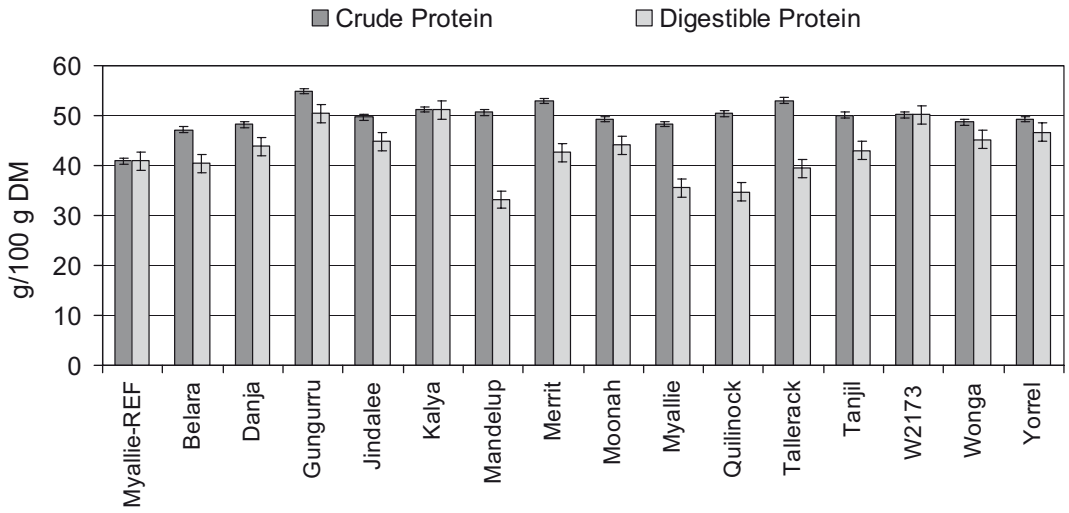

Figure 9. Variability in digestible protein levels among *L. angustifolius* kernel meals.

Preliminary evaluation of the variability in the digestible energy and protein value of the 15 cultivars was examined against key compositional variables of the meals. In contrast to earlier work (Glencross et al., 2003), it was observed that the digestible energy and protein content of the samples was not strongly related to their protein content. Neither were there relationships between these digestibility factors and crude fibre or crude carbohydrates.

However, it is noted that these 15 samples were atypical lupin kernel meals and it is not known how much that this may have influenced key relational factors, such as digestible protein and energy values. Future work will progress this area by increasing the data-set and ensuring that a more realistic range of samples is utilised in the assessment. In 2005 three are plans to examine a further 45 lupin kernel meal samples.

Growth and Palatability

In past years there have been several studies with all three key commercial lupins species, where incremental inclusion levels have been examined (Burel et al., 1998; Farhangi and Carter, 2001; Glencross et al., 2004a). In each of these studies key aspects of fish performance including growth, nutrient and energy retention and feed intake/diet palatability have been examined.

The inclusion of a *L. albus* kernel meal at 300 g/kg, 500 g/kg and 700 g/kg in diets that were designed to be iso-nitrogenous and iso-energetic identified that white lupin kernel meal could be included in the diet of rainbow trout to a level of 500 g/kg with no loss in growth rate and significantly superior phosphorus retention (Burel et al., 1998). The loss in growth performance of fish fed the diets containing 700 g/kg white lupin kernel meal was attributed to low feed intakes of this diet. It was suggested that high levels white lupin kernel meal inclusion resulted in a loss of palatability of the diet.

A study examining the iso-nitrogenous and iso-energetic inclusion of *L. angustifolius* kernel meal at 100 g/kg, 200 g/kg, 300 g/kg, 400 g/kg and 500 g/kg of the diet was also undertaken (Farhangi and Carter, 2001). This study showed that sweet lupin kernel meal could be effectively included in diets for trout at up to 400 g/kg, but that at 500 g/kg there was a significant reduction in growth and a deterioration in feed efficiency. Further examination of the immunological status of the fish showed no aberrations associated even with the highest inclusion levels. Regression analysis of the growth data suggested that there was a significant decline at all inclusion levels.

A similar study examined the iso-nitrogenous and iso-energetic inclusion of *L. luteus* kernel meal at 125 g/kg, 250 g/kg, 375 g/kg and 500 g/kg of the diet (Glencross et al., 2004a). This study showed that yellow lupin kernel meal could be effectively included in diets for trout at up to 375 g/kg, but that at 500 g/kg there was a significant reduction in growth, but no loss in feed intake. Regression analysis of the growth data suggested that there was a significant decline at all inclusion levels.

Recent studies examining the energetic use of diets containing *L. angustifolius* (cv. Myallie) kernel meals at 15% and 30% inclusion show that use of this ingredient does not significantly impair either protein or energy utilisation efficiency. Diets were formulated to equivalent digestible protein and energy specifications and then fed at several ration levels. Examination of the energy/protein retention relative to energy/protein intake allows the determination of utilisation efficiencies for each diet. Energy utilisation efficiency of fish fed these diets was in excess of 80%, where as protein utilisation was 50% efficient. This information supports that utilisation of plant protein/energy is not inferior to that of fish meal protein, provided diets are formulated on a digestible basis.

Anti-Nutritional Factors

Many plants have evolved a range of chemical compounds as a defence mechanism, against being eaten by animals. Legumes, including lupins, have an extensive array of such anti-nutritional factors (ANF), which are also referred to as bioactive compounds. Like other legumes, lupins also have some ANF, although considerably less than plants such as soybean. The key ANF in lupins includes alkaloids and oligosaccharides, although legume allergens and others have also been reported (Table 12).

Table 12. Common anti-nutritional factors found in grain protein resources.

Anti-Nutrient (g/lg DM)	*L. angustifolius* (kernel)	*L. luteus* (kernel)	*L. albus* (kernel)	*L. angustifolius* (whole seed)	Soybean meal (defatted)
Protease Inhibitor	0.20	-	-	0.12	3.11
Alkaloids	0.25	0.08	0.03	0.20	0.01
Oligosaccharides*	68	110	84	41	68
Phytate	5.0	-	-	4.0	15.9
Saponins	0.6	-	-	0.6	6.7
Tannins	-	-	-	0.1	-

*Sum of raffinose, stachyose and verbascose.

Alkaloids

Alkaloids have long been regarded as the key anti-nutritional component of lupins. Selective breeding over the past 50 years has produced cultivars of several lupin species with low levels of alkaloids. Alkaloids are generally bicyclic, tricyclic or tetracyclic derivatives of the molecule quinolizidine. The amount and variety of alkaloids found in the different lupin species also varies. In *L. luteus*, one of the predominant alkaloids is gramine. Notably, current commercial cultivars of *L. luteus* are very prone to insect infestation and this has been shown to be related to their inherent low levels of alkaloids and in particular gramine (Berlandier and Sweetingham, 2003).

In 2004 a study was initiated to examine the response of fish to dietary inclusion of purified gramine (Figure 10). In addition two cultivars of *L. luteus* (cv. Wodjil and Teo) were also examined. The study showed that a critical threshold for gramine tolerance exists between 100 mg/kg and 500 mg/kg. Inclusion of 30% *L. luteus* cv. Wodjil in a diet had no negative effect on feed intake or growth, but inclusion of 30% *L. luteus* cv. Teo in a diet had a significant effect on both feed intake and growth. Negative controls based on a fish meal reference with sodium sulfamerzine inclusion were also used to demonstrate experiment capacity and feed intake effects independent of dietary gramine content. Down regulation of thyroid hormones was also noted, but this appeared to be related to feed intake, not specifically to dietary gramine content.

Histological examination of key organs of the fish from each treatment showed an increased presence of melano-macrophage centres (MMC), with increasing feed intake reduction. No other gramine specific histological aberrations were noted. It was concluded that gramines primary mode of anti-nutritional activity was through its influence on feed intake.

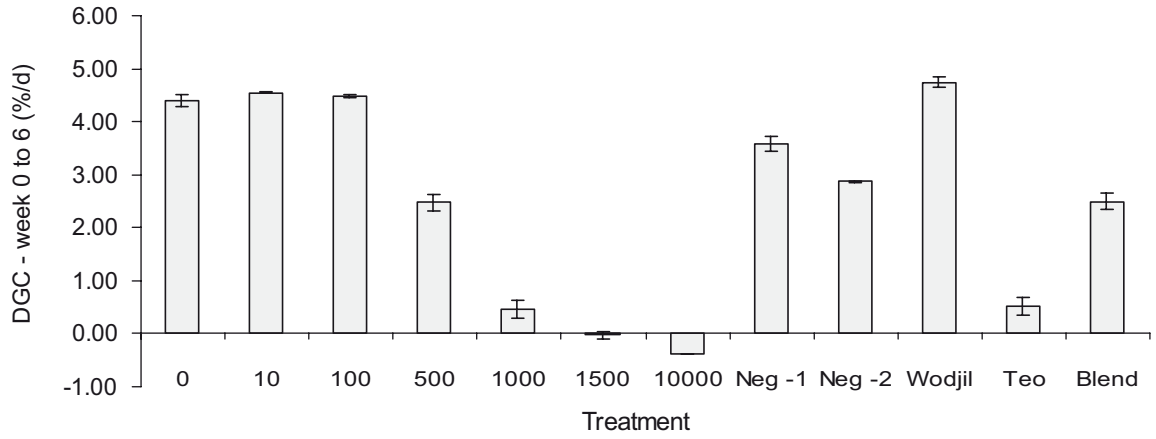

Figure 10. Growth response (daily growth coefficient %/d) of rainbow trout to the inclusion of the gramine and also the response of domesticated and undomesticated lines of *L. luteus* kernel meal.

Oligosaccharides

The oligosaccharides are generally α-galactosyl homologues of sucrose. Oligosaccharides also contain significant amounts of the raffinose, stachyose, verbascose and sucrose families. Of these raffinose has a single galactose moiety linked to a sucrose molecule, while stachyose has two and verbascose three. High levels of raffinose oligosaccharides have been reported to present some negative nutritional effects, some of which may be applicable to fish.

In 2001 a study was undertaken to examine the influence of lupin oligosaccharides on the digestibility of a series of test diets (Glencross et al., 2003). This study used a tandem approach of both enzymatic and chemical methods to remove the oligosaccharides and examine the influence of this removal on the digestibility of the diets.

It was shown that both enzymatic and chemical methods made an improvement in digestion of both protein and energy, but that the effect of chemical extraction was slightly more pronounced (Table 13). Controls for the chemical extraction method and the utilisation of free-galactose (the monosaccharide produced from enzymatic processing) were also included in the study.

Table 13. Digestibility response of rainbow trout to the presence or absence of lupin oligosaccharides. Derived from Glencross et al. (2003).

Digestible nutrient	Kernel meal	Enzyme	Reconstituted	Extracted	Galactose	Pooled SEM
Protein (%)	79.2	85.1	85.8	86.8	87.0	1.22
Energy (%)	51.7	59.3	52.1	63.9	64.4	3.32

Kernel meal: *L. angustifolius* kernel meal (cv. Mixed). Enzyme: *L. angustifolius* kernel meal with 30IU/kg α-galactosidase. Reconstituted: Ethanol (70%) extracted *L. angustifolius* kernel meal with supernatant and precipitate reconstituted. Extracted: Ethanol (70%) extracted *L. angustifolius* kernel meal with supernatant removed. Galactose: Ethanol (70%) extracted *L. angustifolius* kernel meal with equivalent weight of crystalline galactose added as was material removed through extraction.

Legume Allergens

In 2004, Food Standards Australian and New Zealand made a media release on the potential need to introduce warnings on the presence of allergens present in lupins in human food stuffs. Legume allergens have been well known from peanuts and soybean although the specific agent causing the allergy response even in these well known allergenic substances is not clear. This issue also raised the question of the potential impact of lupin allergens in fish.

There is limited literature on allergies in fish, but in mammals such allergies are related to an acute response by immunoglobin E (IgE). However fish do not have IgE's, producing only immunoglobin M (IgM) in acute situations and immunoglobin G (IgG) in chronic situations. It was hypothesised that if fish had a chronic allergy response to the presence of lupins in their diet, then an increase in IgG should occur. With an acute response an increase in IgM should occur.

In conjunction with another trial fish plasma samples were collected from fish fed diets containing only fish meal as a protein source, 15% and 30% *L. angustifolius* cv. Myallie kernel meal. The plasma samples were then electrophoresed and variation in the intensity of the IgG band examined. No differences in IgG band intensity were noted amongst the three treatments and it was concluded that it is unlikely that a chronic immune response to the inclusion of dietary lupin kernel meal exists. Further studies on possible acute IgM responses are still being planned.

Heavy Metals

Like all plants, lupins too absorb and accumulate some minerals from their environment. One group of minerals that have potential negative connotations are the heavy metals (cadmium, mercury, lead). Cadmium in particular is known to accumulate in seeds of *L. luteus* cv. Wodjil and concerns were expressed about the implications of this for its use as a feed grain in the intensive animal industries.

In 2002 a study was undertaken in which incremented inclusion levels of *L. luteus* cv. Wodjil kernel meal were included in standard salmonid formulations. Two additional treatments that included two levels of cadmium chloride were also included. The fish were fed the diets for 6-weeks after which a range of factors, such as cadmium digestibility, heavy metal/nutrient/energy retention and organ histology were examined.

Cadmium accumulation was related to total dietary cadmium content, but was not related to the inclusion of *L. luteus* cv. Wodjil kernel meal. Subsequent investigation of the cadmium level of other feed ingredients showed that *L. luteus* cv. Wodjil kernel meal was lower in cadmium than fish meal. Cadmium digestibility was generally low (11 – 49%) and showed no direct relatiojnship to either lupin or fish meal inclusion levels. Gross cadmium retention was also low (< 3%). Of the cadmium that was accumulated the highest concentrations were in the kidney and liver of the fish. Greatest cumulative amount was in the muscle, due to its high proportion of the total body weight, and also the skin.

Protein Concentrates

Protein concentrate development for application in the aquaculture feeds sector has been undertaken since the mid 1990's, but in earnest since 2002 when the Grains R&D Corporation sponsored a project to specifically examine this potential. One of the key limitations in using lupin products in aquaculture feeds was their low-mid protein level and the high level of non-nutritionally useful carbohydrates present in the grain. Initially the key objective for the present project, protein concentrates were developed from both *L. angustifolius* and *L. luteus* varieties. Prototype materials were first developed in the laboratory using protein isolation technology (Sipsas, 2003). These were evaluated in fish trials in 2003 (Glencross et al., 2004b; 2005).

All of the protein concentrate (PC) products proved to have high levels of digestible protein and energy. Little differentiation in digestible quality was noted between the two lupin varieties. However, *L. luteus* enabled the development of a protein concentrate with substantially higher protein levels

Subsequent evaluation examined the palatability of the protein concentrates and their utilisation when fed to rainbow trout. These studies showed that there were no long-term palatability issues with either PC, but that high (40%) inclusion of the *L. angustifolius* PC did cause a significant reduction in feed intake during the first ten days. However, this poorer initial feed intake did not impact on growth of the fish. No significant effects of either PC on nutrient/energy retention were noted.

Following the proving of the lab-scale potential for PC's as an aquaculture feed ingredient, production was up-scaled to allow the assessment of commercial drying technologies. The drying costs and yield factors were identified as the two most cost-sensitive points influencing the potential viability of commercial development of PC's from lupins (Kingwell, 2003).

Several 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 getting the opportunity to use the facilities at a reasonable cost proved difficult. In the end, our liquid concentrates were freighted to Saurin Technologies Pty Ltd, in the Dandenong region of Victoria, who have a pilot-scale industrial process drying facility. At their facility we managed to test the viability of both spray-drying and ring-drying.

The spray-drying technique proved very useful in drying the liquefied concentrates. Few problems were encountered in the processing, and several hundred kilograms of product was produced. However, ring-drying proved problematic with the liquefied concentrate in-feed part of the process. On introduction of the liquefied concentrate, it tended to clump, and form rubberised-like conglomerates, making the ring-drying process ineffective. Further pursuit of this drying technology was abandoned to these problems and time constraints.

To examine the nutritional limitations to the influence of drying technologies on the production of lupin protein concentrates, two additional drying techniques were also evaluated. Samples of the same wet concentrate were also freeze-dried and also oven dried at 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 14. 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., 2004c).

Table 14. Composition of 2nd-stage prototype ingredients evaluated. Details are on a dry matter basis (g/kg DM) unless otherwise specified.

INGREDIENTS	MKM	MPCD	MPCF	MPCS	LKM	LPCD	LPCF	LPCS
Dry Matter (g/kg)	912	953	932	937	918	945	917	939
Protein	409	734	744	764	543	770	793	811
Fat	68	88	130	86	73	70	109	86
Ash	37	39	30	27	44	43	40	31
Phosphorus	5	7	7	6	6	8	7	7
Energy (MJ/kg DM)	20.4	24.0	23.9	24.3	21.1	24.6	24.8	25.0

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.

Since produced, these products have been evaluated for their digestible nutrient and energy value and their utilisation when included in salmonid diets. Methods used were similar to those of earlier studies were we used the diet substitution approach of Aksnes et al., (1996) and calculations of Sugiura et al., (1998). Digestibility specifications showed that the oven drying did not reduce the digestible value of the products (Table 15). This was in contrast to what was seen earlier for oven drying/heat damage of canola meals (Glencross et al., 2004c).

Table 15. Apparent digestibility coefficients of 2nd-stage prototype diets and test ingredients.

	Ref	MKM	MPCD	MPCF	MPCS	LKM	LPCD	LPCF	LPCS	EHC
Diet Digestibility										
AD Energy	0.810	0.808	0.858	0.846	0.866	0.833	0.805	0.818	0.857	0.864
AD Protein	0.900	0.907	0.930	0.914	0.904	0.888	0.924	0.895	0.916	0.917
AD Lysine	0.911	0.906	0.928	0.922	0.924	0.905	0.919	0.914	0.927	0.955
AD Methionine	0.963	0.963	0.966	0.952	0.947	0.948	0.971	0.947	0.955	0.970
AD Arginine	0.938	0.948	0.966	0.960	0.948	0.938	0.964	0.937	0.959	0.903
AD Threonine	0.923	0.916	0.934	0.922	0.912	0.904	0.930	0.894	0.918	0.937
AD Valine	0.942	0.939	0.959	0.943	0.928	0.925	0.957	0.908	0.937	0.960
Ingredient Digestibility										
AD Energy		0.762	0.969	0.927	0.916	0.774	0.680	0.783	0.903	0.944
AD Protein		1.020	1.038	0.965	0.959	0.887	1.041	0.924	1.001	0.918
AD Lysine		0.863	1.174	1.099	1.087	0.906	11.323	3.942	1.040	1.037
AD Methionine		2.330	1.066	1.046	0.681	1.881	1.233	1.071	1.157	1.010
AD Arginine		1.059	1.086	1.082	1.011	0.967	0.902	0.899	1.017	0.845
AD Threonine		1.030	1.095	1.006	0.900	0.886	0.858	0.729	0.951	1.142
AD Valine		1.055	1.119	1.148	0.985	0.998	48.014	0.785	0.956	1.107

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.

Evaluation of the utilisation value of the protein concentrates dried using either oven-drying (heat damaged: MPC-Oven Dried) or spray-drying (MPC-Spray Dried) was undertaken to determine if there was any reduction in the ability of fish to utilise energy or protein from the protein concentrates.

The test ingredients were included at 30% in diets formulated on equivalent digestible protein (373 g/kg DM) and energy basis (17.0 MJ/kg DM). The diets were fed at various ration levels to replicate (n=4) tanks of 20 fish for 28 days. After 28 days the fish were weighed and samples collected for analysis.

Examination of energy retention of the three diets showed that the MPC-Oven Dried was significantly poorer utilised than the other two diets. Further evaluation of the ammonia/urea content of water samples from those treatments showed that a significantly higher proportion of nitrogen was being excreted as urea by fish in the MPC-Oven Dried treatments. This explains the relative loss of energy value by the fish fed these diets.

The protein retention by fish fed these diets also shows two clear features. Firstly, the limitation in amino acid composition of the MPC-Spray Dried is showed by the lower protein retention at the higher protein intake rates. That energy retention was equivalent to the fish meal diet at these higher intake rates supports than there must be differences in the form of energy retention between the two treatments. The poorer available protein to metabolisble energy ratio of the MPC-Oven Dried is showed by its still lower protein retention at the higher intake rates compared to the MPC-Spray Dried. It is interesting that at lower protein intake rates that there is limited difference in the efficiency of the utilisation of protein among all three treatments.

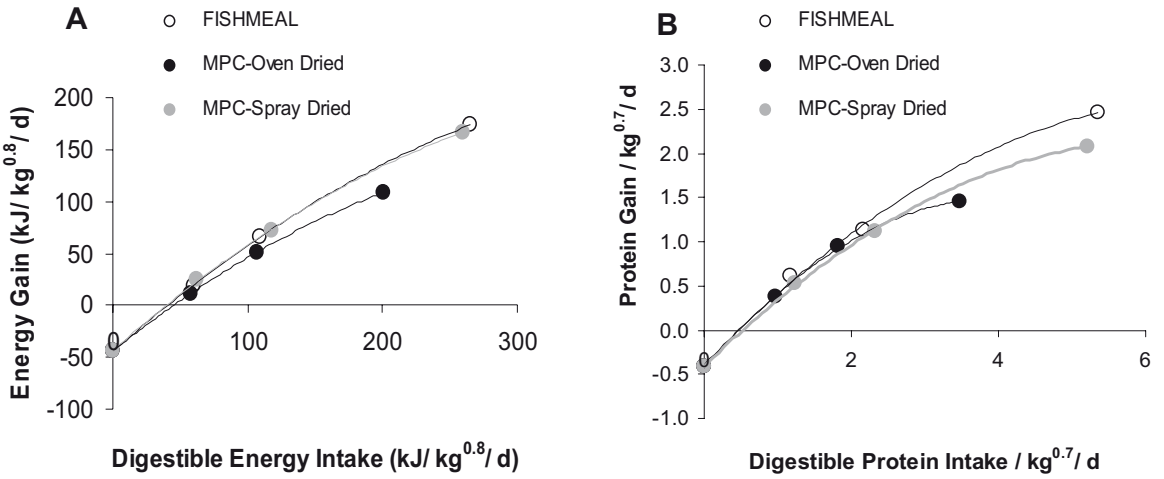

Figure 11. Energy (A) and protein (B) retention with variable intake levels. Departure of lines from the reference (Fishmeal) treatment shows differential utilisation efficiency.

Progress on further PC development for the aquaculture feed sector has been stalled to allow greater focus on lupin kernel meal development. However, some work is still being maintained in the area of PC development through applications for human foods and within the commercial sector. The program will continue to assess the commercially supplied PC's, but public release of data pertaining to these will be at the commercial sector's discretion.

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Different lupin meals and concentrates to Atlantic salmon at low temperature: Digestibility and intestinal physiology

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Introduction

Australian lupin kernel meals are increasingly being used in feeds for Atlantic salmon as a supplement or alternative to soybean meals. This is due to high protein content, but also because of potentially less problems with antinutritional factors (ANFs).

Dietary soybean meals induce enteritis and reduce the hydrolytic activity of brush border membrane bound enzymes in the distal intestine of Atlantic salmon (Baeverfjord et al., 1996; Ingh et al., 1996; Refstie et al. 2001, 2005; Krogdahl et al., 2003). This coincides with reduced overall digestibility of lipid (Refstie et al., 2001, 2005; Krogdahl et al. 2003). Ethanol extraction of soybean meals to produce soy protein concentrates largely eliminates distal enteritis and reduced lipid absorption in salmon (Ingh et al., 1996; Refstie et al. 2001).

Both lipid and protein digestibility in salmon is furthermore affected by water temperature (Bendiksen et al., 2003). Furthermore, the lowering in lipid digestibility when feeding soybean meal appears to be worsened at low ambient temperature (Refstie et al., 2001). Thus, it is important to evaluate the digestible value of lupin products and lupin containing feeds at different temperatures to increase our understanding of how these ingredients are best utilised.

Such aspects have not been investigated when feeding lupin products to salmon. Thus, the present work evaluated five lupin products developed as part of the linked CLIMA-GRDC project, as well as extracted soybean meal (SBM). The lupin products were dehulled lupin kernel meal from *L. luteus* cv. Wodjil (LKM), *L. a.* cv. Belara (high viscosity; BKM), and *L. angustifolius* cv. Myallie (low viscosity; MKM), and spray dried lupin protein concentrates made from the above *L. l.* (LPC) and *L. a.* cv. M (MPC). Composition of the ingredients is given in Table 16. Our objectives were 1) to estimate digestibility of nutrients in these lupin products and compound diets containing the lupin products in salmon kept at low (~6°C) temperature, and 2) to study how the lupin products affected digestive function and intestinal pathology in the salmon when compared to feeding extracted soybean meal.

Table 16. Composition of the test ingredients.

Diet code	FM	SBM	LKM	BKM	MKM	LPC	MPC
Dry matter, g kg^{-1}	931	913	918	918	921	939	937
In DM							
CP*, g kg^{-1}	749	531	543	452	452	811	764
Lipid, g kg^{-1}	87	15	73	80	73	63	86
Carbohydrates, g kg^{-1}	3	386	340	434	441	95	122
Organic matter, g kg^{-1}	839	932	956	966	967	969	973
Energy, MJ kg^{-1}	20.5	18.9	21.1	20.2	20.3	24.3	25.0

*Crude protein, N x 6.25.

Experimental conduct

A basal (+ve control) diet was formulated to contain 700 g fish meal, 150 g fish oil, 144 g wheat, 5 g vitamin and mineral pre-mix, and 1g yttrium oxide per kg dry diet (**FM**). The dry ingredients in this diet formed the basal mix for five experimental diets, which were formulated to contain 595 g basal mix, 300 g lupin, and 105 g fish oil per kg diet. A similar diet was formulated with 300 g SBM per kg (–ve control). All diets were manufactured by high-pressure moist extrusion at the Australian Experimental Stockfeed Extrusion Centre (Roseworthy College, S.A.) using a Wenger X185 experimental scale extruder. Following extrusion each diet was dried to about 6% moisture content prior to coating with fish oil.

The experiment was done at AKVAFORSK (Institute of Aquaculture Research) in Sunndalsøra, Norway. When initiating the experiment, 121 fish with a mean body weight of 176 g were randomly allocated to each of 21 tanks supplied with 5.6°C saltwater. Each diet was then fed to 3 groups of fish for a period of 21 days prior to stripping of faeces and sampling of plasma and gastrointestinal tissues. The test ingredients and diets were analysed for dry matter, nitrogen, lipid, ash, and gross energy (adiabatic bomb calorimetry). The diets were furthermore analysed for starch and yttrium. The faeces were freeze dried and analysed for the same except starch. Organic matter and carbohydrates were calculated by difference. Analyses of individual amino acids in ingredients, diets, and faeces are pending.

Digestibility of nutrients in the different dies was estimated by the indirect method, using yttrium oxide as the inert marker (Austreng et al., 2000). Digestibility of nutrients in the different lupin products and the SBM were assessed by the diet-substitution method (Aksnes et al., 1996), as previously described by Glencross et al. (2004).

Blood was taken from 10 individual fish per tank, and the plasma was immediately frozen in liquid nitrogen for analyses of plasma chemistry. Intact gastrointestinal tracts were removed from the same fish, immediately frozen in liquid nitrogen, and stored at -80°C for analyses of luminal enzyme activities. Segments of stomach, pylorus intestine, mid intestine and distal intestine of 6 fish per tank were rinsed and immediately frozen in liquid nitrogen, and stored at -80°C for analyses of brush border membrane bound enzyme activities. Segments of stomach, pylorus intestine, mid intestine, distal intestine, liver, spleen, thymus, and head kidney of 2 fish per tank were rinsed and preserved in phosphate-buffered formalin for histological examination. Segments of stomach, pylorus intestine, mid intestine, distal intestine, liver, and adipose pyloric tissue containing pancreatic tissue from 3 fish per tanks (omitting the tanks fed LPC and MPC) were rinsed in PBS, frozen in liquid nitrogen, and stored in RNA later ICE for micro array analyses of gene expressions in response to diet.

Preliminary results

Table 17. Apparent digestibility of nitrogen, organic matter, and energy in the tested diets, and faecal dry matter (DM) content when feeding the diets.

Diet code	FM	SBM	LKM	BKM	MKM	LPC	MPC
Digestibility (%) of							
Nitrogen	83.6^{b}	84.0^{b}	84.3^{b}	84.9^{b}	83.8^{b}	87.9^{a}	88.1^{a}
Organic matter	74.1^{b}	69.0^{cd}	70.4^{c}	68.2^{d}	67.4^{d}	78.6^{a}	79.6^{a}
Energy	79.2^{b}	75.1^{c}	77.2^{bc}	76.6^{bc}	76.1^{c}	82.6^{a}	84.6^{a}
Faecal DM content, %	15.5^{a}	10.7^{e}	14.4^{b}	13.3^{d}	14.3^{bc}	13.5^{cd}	13.7^{bcd}

Different superscripts within a row denotes differences ($P<0.05$) as indicated by ANOVA and Duncan's multiple range test.

The digestibility of nitrogen, organic matter, and energy when feeding the different diets are shown in Table 17. The nitrogen digestibility was highest when feeding the diets with lupin protein concentrates. The nitrogen digestibility was similar when feeding the other diets. The digestibility of organic matter and energy was generally lowest when feeding the diets with SBM or lupin kernel meal. However, as each diet contained 30% test ingredient, these results are influenced by the different contents of nutrients and indigestible carbohydrates in the different ingredients (Table 16).

The faecal dry matter content was highest when feeding the FM diet (Table 17). Dietary SBM resulted in watery faeces, as seen from the low faecal dry matter content. This is typically seen when feeding soy to salmon (Refstie et al., 2001, 2005). Dietary BKM (high-viscosity *L. a.*) resulted in more watery faeces than dietary LKM and MKM (low-viscosity *L. a.*), while both diets with lupin protein concentrates gave intermediate faecal dry matter.

When estimated for the ingredients, the digestibility of nitrogen, organic matter, and energy was significantly higher in both lupin protein concentrates than in the SBM and lupin kernel meals (Table 18). The nitrogen digestibility was generally lowest in the lupin kernel meals, and intermediate in the SBM. When comparing the lupin kernel meals, the nitrogen digestibility was highest in the BKM and lowest in the MKM. However, the digestibility of organic matter was higher in the LKM and SBM than in the MKM, with an intermediate estimate for the BKM. This was probably caused by higher nitrogen and, thus, crude protein content in the LKM and SBM. The digestibility of energy was similar in the SBM and lupin kernel meals, but the ranking of numerical figures corresponded with the differences in nitrogen and organic matter digestibility.

Table 18. Apparent digestibility of nitrogen, organic matter, and energy in the test ingredients.

Ingredient	SBM	LKM	BKM	MKM	LPC	MPC
Digestibility (%) of						
Nitrogen	91.1 ^{c}	79.4 ^{e}	84.8 ^{d}	70.5 ^{f}	107.7 ^{a}	96.8 ^{b}
Organic matter	61.6 ^{b}	59.1 ^{bc}	53.7 ^{cd}	50.3 ^{d}	92.7 ^{a}	92.1 ^{a}
Energy	72.2 ^{b}	69.7 ^{b}	72.3 ^{b}	67.9 ^{b}	92.3 ^{a}	94.6 ^{a}

Different superscripts within a row denotes differences ($P<0.05$) as indicated by ANOVA and Duncan's multiple range test.

The digestibility of nitrogen in the LPC was estimated at >100%. Similar estimates were previously found by Glencross et al. (2004) when evaluating several lupin products in Atlantic salmon and rainbow trout. As then, we have not corrected this because we believe it is potentially indicative of interactive effects between diet and test ingredient. However, when calculating the level of digestible nitrogen in the ingredients (Table 19), we assumed the nitrogen digestibility in the LPC to be 100%.

The results given here are preliminary. All analytic results and calculations will be checked upon final publication of the figures. Furthermore, results on lipid digestibility in the diets, amino acid digestibility in diets and ingredients, as well as studies of digestive function and intestinal pathology are pending.

The studies of digestive function and intestinal pathology will be done in collaboration with the Gut Health Group of the Aquaculture Protein Centre (APC). It is unknown if lupin kernel meals cause the same enteritis problems in the distal intestine of Atlantic salmon. Thus, this comprehensive histological and enzymological approach will clarify to what extent possible antigenic influences of lupin kernel meals are problematic when fed to Atlantic salmon.

Table 19. Digestible nutrient content in the ingredients when fed to Atlantic salmon at 5.6°C.

Ingredient	SBM	LKM	BKM	MKM	LPC	MPC
In DM						
CP*, g kg^{-1}	483	431	383	319	811	740
Organic matter, g kg^{-1}	574	565	519	486	898	896
Energy, MJ kg^{-1}	13.7	14.7	14.6	13.8	22.4	23.7

*Crude protein, N x 6.25.

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Apparent digestibility and gastrointestinal transit of lupins in Atlantic salmon

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Introduction

This experiment aimed to determine the apparent digestibility of a reference soybean meal, three lupin kernel meals and a lupin protein concentrate and compare these to gastrointestinal evacuation rates in seawater Atlantic salmon.

Materials and Methods

Fish

All female pre-smolt Atlantic salmon were obtained from the Huon Aquaculture Company (Tasmania, Australia) over 3 weeks (farm weight estimate, 493 ± 42 g). Fish were held at the School of Aquaculture in six 2000-L Rathbun tanks that were part of self-contained partial recirculation systems equipped with physical, biological and UV filtration. The fish was acclimated to the systems that were used to hold the fish for the experiments. Water temperature was controlled at 15.0 ± 1.5 °C and fish were exposed to ambient photoperiod. Water quality was maintained within recommended limits for the experiments (Wedemeyer 1996). A commercial salmon feed was hand fed 2-3 times per day for 4 to 6 weeks.

Diets

A reference mash was formulated and 5 experimental diets made to include 30% of each test ingredients. The reference mash contained 0.1% Yttrium oxide as an inert digestibility marker. Ingredients tested were *L. luteus* protein concentrate (LPC), *L. luteus* (cv Wodjil) kernel meal (LKM), *L. angustifolius* (cv. Belara) kernel meal (BKM), *L. angustifolius* (cv. Myallie) kernel meal (MKM), soybean meal (SBM).

Apparent Digestibility (AD)

At the start of the apparent digestibility experiment all diets were hand fed three times a day to appetite and feed intake estimated from the weight of pellets fed. The six diets were randomly allocated to one group in each of three time periods. Diets were fed for 7 days and the fish stripped (Austreng 1978; Percival et al. 2001) on day 8 in the morning. In order to randomise the effects of previous diets the fish were re-mixed during re-allocation to tanks. Groups were fed the commercial feed for 6 to 7 days and then transferred to the experimental diet for a further 7 days. Following initial sampling fish were reused twice to obtain triplicate samples for each diet. Faecal samples were freeze dried pooled into one sample per tank and one sample per tank analysed for each of Yttrium, crude protein, crude fat, phosphorous, ash and gross energy.

Gastric Evacuation Time (GET)

The intention was to measure GET at the end of the AD experiment. The procedure would be for the fish to do through the same sequence as between measures for AD. Fish re-allocated to new groups and fed the commercial diet for 6 d and experimental diets fed for a further 7 d. On day 8 a diet labelled with ytterbium fed at the first feed and the usual yttrium diet fed as normal there after over the next 72 h. Three fish from each tank to be removed following feeding at 0.5, 3, 6, 12, 24, 48 and 72 h. Fish killed by a blow to the head and the weight and length measured. The gastrointestinal tract was removed and the stomach, pylorus, mid gut and hind gut ligated (Sveier et al. 1999). The sections frozen, the frozen contents removed and weighed and then analysed individually for dry material, crude protein, yttrium and ytterbium. This experiment was not conducted due to a shortage of fish.

Table 20. Formulation of experimental Atlantic salmon feeds containing different plant protein ingredients

	REF	LKM	LPC	BKM	MKM	SBM
Fish meal	700	490	490	490	490	490
Fish oil	150	150	150	150	150	150
Wheat flour	150	105	105	105	105	105
Pre-mix vitamins	5	5	5	5	5	5
Yttrium oxide	10	10	10	10	10	10
Plant protein		300	300	300	300	300

A replacement GET experiment is suggested and based on a faecal collection method that involves the replacement of one marker with a second marker in the same feed (Storebakken et al. 1999). The two feeds supplied were of different pellet size so they will first be re-pelleted to uniform size. Fish will be held in 350-L conical bottomed tanks fitted with Guelph type faecal collectors (Carter and Hauler 2000). Feeds will then be fed to Atlantic salmon smolts (100 g) for 7 days (or until high feed intake is reached). Faecal samples will be taken at different times following introduction of the second feed, probably at 6-hourly intervals, over the following 72 hours. Faecal samples will be analysed for Yttrium and Ytterbium to investigate the time course of 100% replacement of the first marker by the second marker.

Results and Discussion

Seawater Atlantic salmon (500-1000g) were successfully transferred to the School of Aquaculture, maintained in 2000-L tanks and consumed the experimental feeds containing 30% lupin based protein sources. These fish were from commercial cages and took time to acclimate to the indoor tanks. Initial feed intake was relatively low and there were marked size related differences in feed intake. Future experiments will aim to use salmon grown through the Aquaculture Centre when possible.

At this time no data on chemical composition are available so that digestibility cannot yet be calculated.

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Adapting lupins for use in prawn diets – Benefits and constraints

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Introduction

There has been considerable interest in the use of lupins in aquaculture feeds over the last 12 years. The earlier work, which started in 1993, focused on the narrow-leafed lupin (*Lupinus angustifolius,* var Gungurru*)* with a small amount of work on the white lupin (*L.* albus) and the yellow lupin (*L. luteus)*. More recently, as part of the CLIMA program, newer varieties of lupins have been investigated.

The lupin products that have been evaluated in aquaculture feeds include meals prepared from both whole seed and dehulled seed (lupin kernel meal). In addition, a lupin protein concentrate, prepared by air classification of kernel meal from *L. angustifolius,* cv. Gungurru, has been evaluated in a number of studies (Carter and Hauler, 2000; Sarac *et al.*, 1998; Sudaryono *et al*., 1999a,b; Booth *et al.*, 2001). More recently a protein concentrate, prepared through aqueous ethanol extraction of lupin kernel meals, has been evaluated in feeds for rainbow trout. (Glencross *et al.*, 2003).

Lupin meal is included in a prawn feed formulation primarily to provide protein or essential amino acids. A secondary consideration is the digestible energy that it contributes to the formulation. The other nutrients contributed by the lupin meal to the feed as a whole are of lesser importance. However, any negative attributes, such as components that adversely affect digestibility or are anti-nutritional factors, are of greater significance. Hence, an 'ideal' lupin meal would have a high protein content, good digestibility and no anti-nutritional factors.

Research into the use of lupin meals in prawn feeds has focused on determining the digestibility of lupin meals, the effect inclusion level of lupins has on the growth response of prawns, and identifying the factors that limit the use of lupins in the feed. When evaluating new lupin products, or the attributes of new cultivars, consideration must be given to the attributes of the other feed ingredients, particularly fishmeal and soybean meal, against which the lupin products will be competing for inclusion in the feed formulation. In this paper I highlight the benefits and constraints of lupin meals as ingredients for prawn feeds in order to identify the characteristics that warrant further development to improve their efficacy in these feeds. In this paper, all information on chemical composition of ingredients are stated on a dry matter basis.

Chemical composition

Typically, lupin seeds have higher crude protein content (31 to 42 %) than most other grain legumes. There is considerable variation in protein content among species and cultivars and even within cultivars. Of the commercial species, *L. luteus* is generally considered to have the highest protein content of whole seed (40 to 45% DM), while the kernel typically contains about 53% (Table 21, Petterson *et al*. 1997; Sipsas, 2003). The whole seed of *L. albus* is also high in protein, having a protein content of between 32 and 44%, and the kernel having about 44%. The protein content of *L. angustifolius* seed typically ranges from 30 to 41% DM, with the kernel having about 44%. In comparison, dehulled, solvent-extracted soybean meal has a crude protein content of about 53%. The crude protein content of fishmeal is highly variable, but high quality Peruvian fishmeal (Super prime) provides a widely-accepted yardstick with about 75% protein.

The amino acid composition of lupin protein is similar to that of soybean but is characterised by relatively high levels of arginine, 11.3 to 12.2 g/16 g N, which is about twice the level in soybean protein. However, it has relatively low levels of methionine, 0.65 g/16 g N, or about half that of soybean protein. Hence, the total sulphur amino acid content (methionine+cysteine) is also low, 2.1 g/16 N. In prawn feeds with a protein content of

~40% 'as used', the recommended amount of methionine is 0.96% (Akiyama *et a*l. 1991). This is met in a prawn feed formulation with about 320 g/kg fishmeal and 100 g/kg lupin kernel meal. However, the stated requirements appear to be very conservative, as diets with 40% crude protein and ~200 g/kg lupin kernel meal have performed as well as a basal diet that contained no lupin protein but additional fishmeal (Smith, 2002). This is clearly an issue that needs to be resolved.

The crude fat content of lupins also varies considerably among species and cultivars. Of the commercial species, *L. luteus* generally has the lowest fat content, from to 5.7 to 6.8 %, and *L. albus* the highest, 8.3 to 14.5% (Petterson *et al.* 1997; Petterson, 2000; Table 21). The fat in lupins comprises predominantly triacylglycerides (71.1%) and phospholipids (14.9%), with lesser amounts of free sterols (5.2%), glycolipids (3.5%) and other lipid material (5.3%) (van Barneveld, 1999; Petterson, 2000). The crude fat content is significantly higher than in solvent extracted soybean meal (Table 21).

The fatty acid composition of the lipid in lupins is typical of that of most legumes and not unlike that of the residual lipid in soybean meal. It is high in mono-unsaturated fatty acids (MUFA) and poly-unsaturated fatty acids (PUFA), mainly linoleic acid and linolenic acid, but with none of the nutritionally-important, long-chain omega-3 fatty acids found in marine oils. The replacement of fatty acids contained in the fishmeal with fatty acids that are a component of the lupin meal does not have a detectable effect on the performance of prawns when the diet contains 300 g/kg lupin kernel meal (Smith, 2002).

Table 21. Gross chemical composition (% DM) of whole seed and kernel of two species of lupins (Derived from Sipsas, 2003) and Peruvian fishmeal and solvent extracted soybean meal.

Species	*L. angustifolius*		*L. luteus*		Fishmeal	Soybean
	Seed	Kernel	Seed	Kernel		(solvent)
Moisture*	9	12	9	12	9	90
Seed coat	26	–	30	–	–	–
Protein	35	43	42	57	75	53
Fat	7	8	6	8	13	2
Ash	3	3	3	5	14	6
Lignin	1	1	1	1	–	–
Polysaccharides	25	32	9	11	–	26
Oligosaccharides	5	7	10	14	–	13

* % 'as received'

The carbohydrate composition of lupins is quite different from that of most legumes as it contains high levels (> 50% whole seed) of non-starch polysaccharides (NSP) and very low levels of starch (van Barneveld, 1999, Petterson, 2000). In whole seed and in kernel meal, insoluble NSP (or dietary fibre) predominates with lesser proportions of soluble NSP and free sugars. The seed coat is comprised mainly of insoluble NSP, and hence the kernel meal has markedly less NSP than whole seed meal. Nevertheless, the kernel meal typically contains about 40% NSP, but can contain up to 50% NSP. The NSP does affect digestibility. In a series of prawn feeds where insoluble NSP from lupins was added in incremental amounts, the digestibility of the feeds decreased with increasing amount of NSP (Smith, 2002). However, the insoluble NSP did not affect the growth rate of the prawns. The prawns appeared to compensate for the reduced digestibility of the feed by increasing feed intake. The soluble NSP in lupins are predominantly oligosaccharides. These could be considered anti-nutritional factors. The partial extraction of the oligosaccharides from lupin kernel meal with 80% ethanol did not improve the performance of the lupin meal when it was incorporated into a prawn feed. However, this issue has not been adequately addressed.

There is no information available on the response of prawns to the other anti-nutritional factors such as alkaloids, saponins and tannins, which are generally present at low concentrations in varieties of lupins grown in Australia. In South America, kernel meal, prepared from the pearl lupin (*L. mutabilis*), is treated to reduce the concentration of the alkaloid lupanin from 3% to 0.05% before use in prawn feeds (pers. comm. C. Molina, information supplied by Ecuadorian Institute for Agriculture).

Digestibility

The apparent digestibility of lupin seed meal and lupin kernel meal (*L. angustifolius* cv. Gungurru) was determined by Smith *et al*. (1998) in a study with 10 to 15 g prawns (*Penaeus monodon*). The lupin whole seed meal had a significantly lower digestibility than kernel meal. The Apparent Dry Matter Digestibility (ADMD) of seed meal was 39% while that of the kernel meal was 73%; the Apparent Crude Protein Digestibility (ACPD) was 88% and 95%; respectively, and Apparent Digestibility of Energy (ADE) was 45% and 74%, respectively. From the digestibility data, the kernel meal appears to be to be a much better ingredient than the whole seed meal for use in prawn feeds.

In another study, Smith (2002) compared the digestibility of a series of diets containing a lupin protein concentrate (prepared by air classification) with a series containing kernel meal (*L. angustifolius* cv. Gungurru). The kernel meal was included at 200, 400 and 600 g/kg of feed, while the protein concentrate was included at 143, 286 and 429 g/kg, providing the same amount of protein as the kernel meal at each inclusion level. The lupin products were included in the feed formulations at the expense of fishmeal and wheat flour which were adjusted to balance the formulation and maintain the protein content constant. This study confirmed the high digestibility of crude protein in lupin kernel meal. However, the ADMD and ACPD of feeds containing protein-equivalent inclusions of protein concentrate were significantly greater than that of the feeds containing kernel meal (Table 22). This highlights the need to assess whether a feed containing lupin protein concentrate would be more cost effective than one containing lupin kernel meal: whether the enhanced digestibility would offset the additional cost involved in making the lupin protein concentrate.

Table 22. Apparent dry matter digestibility (%) (ADMD) and apparent crude protein digestibility (%) (ACPD) of shrimp diets containing incremental amounts of lupin kernel meal, and lupin protein concentrate from *L. angustifolius*, included in the basal diet (0%) at the expense of fishmeal and wheat flour (Smith, 2002).

Inclusion level	Kernel meal		Protein concentrate	
(%)	ADMD	ACPD	ADMD	ACPD
0	68	82	68	82
20	69	84	68	82
40	62	85	70	86
60	60	86	74	90

In a recent study, we have determined the ADMD, ACPD and ADE of kernel meal produced from a number of varieties of lupins currently under cultivation in Western Australia. Though the ACPDs were similar to that found in the original study with Gungurru (range 93 to 97%, cf. 95%), the ADMDs were about 10% lower. A sample of Gungurru was included in the study, though it was not from the same source as used in the original study. The ADMD of the Gungurru was the highest of the kernel meals in the recent study but was 7% less that that of the original Gungurru. The reason for the difference is not apparent. No correlation has been found between the ADMD, ACPD and the chemical composition of the kernel meals (crude protein, crude fibre, total dietary fibre, insoluble dietary fibre, soluble dietary fibre). Further analyses of the NSP component are underway so that we can examine if there is a correlation between digestibility and either Acid Detergent Fibre or Neutral Detergent Fibre. A key outcome of this work would be to find a component of the kernel meal that correlates well with the apparent digestibility. This information could be used in deciding the most desirable characteristics of cultivars for use in aquaculture feeds.

Growth response studies

The growth of prawns given feeds containing various species and cultivars of lupins has been the subject of a number of studies. Sudaryono *et al*. (1999a) evaluated *L. albus* kernel meal in diets for *P. monodon* containing 400 g/kg crude protein. The lupin kernel meal replaced 0, 25, 50, 75 and 100% of the fishmeal protein with an equivalent of amount of lupin protein. The inclusion levels of the kernel meal in the feeds were 0, 100, 200,

300 and 400 g/kg of feed. Re-analysis of their data demonstrated a progressive decline in weight gain when more than 100 g/kg kernel meal was included in the feed.

Sudaryono and co-workers (1999b) also compared soybean meal with *L. albus* meal in a growth response experiment. Kernel meal was used to incrementally replace solvent extracted soybean meal in a series of feeds containing 40% crude protein (DM basis). The feeds contained fishmeal, squid meal and shrimp meal, which together comprised 360 g/kg of the feed. The soybean meal comprised 300 g/kg of the basal feed. Their data shows a progressive decrease in growth rate with increasing replacement of the soybean meal. This demonstrates that the *L. albus* kernel meal was nutritionally inferior to the soybean meal.

Smith (2002) found that growth rate of prawns was not affected by the inclusion of *L. angustifolius* cv Gungurru kernel meal in feeds at levels up to 200 g/kg. However, at inclusion levels >200 g/kg the growth rate decreased as the kernel meal content of the feed increased. The low level of methionine, or methionine + cystine, in the lupin protein did not appear to limit the nutritional value of the feed when the dietary lupin inclusion level was less than about 380 g/kg. However, this aspect was not tested adequately and requires the development of an effective method for supplementing the diets with methionine before it can be resolved.

Recent studies with cultivars of *L. angustifolius* currently being grown in Western Australia have indicated that these cultivars perform better than the Gungurru used in the earlier studies (ca 1995). Additional experiments are underway to confirm these findings and to compare the performance of the cultivars with solvent extracted soybean meal.

Pellet Stability

When preparing feeds using laboratory equipment, increasing the amount of *L. angustifolius* kernel meal in a feed resulted in a progressive decrease in water stability (defined as the amount of DM retained in the pellet after 4 h immersion in water - Smith, 2002). This effect on feed pellet stability has also been reported with *L. albus* meal (Sudaryono *et al.*, 1999a) and with soybean meal (Lim and Dominy, 1990). Increasing the amount of water added to the dry ingredients during processing improves the pellet stability of the feeds with high lupin meal content, but the extruded strands were too sticky and very difficult to separate. There does not appear to have been a study on the effect of lupin kernel meal on the commercial manufacture of prawn feeds. It is possible that the functional properties of lupin kernel meal will contribute positively to the steam pelleting of the feeds and so enhance pellet stability. However, research into this aspect of lupin efficacy has yet to be carried out and would require a collaborative research project involving a feed manufacturer.

Conclusions

Because lupin meals are included in prawn feeds primarily as a protein source, high protein content and low levels of NSP, which affect protein digestibility, would be considered favourable characteristics. However, though a product with a protein content >50% would appear attractive, the cost of this product may make it economically non-viable. In assessing lupin products, it is important to compare their cost with soybean meal, in terms of the cost per unit weight of protein. The issue of the low methionine content of lupin protein has not been addressed adequately and until it is resolved this may unfairly restrict the use of lupin meals to low inclusions levels in prawn feeds.

Though insoluble NSP has not affected the growth of prawns, it does reduce protein digestibility. So, processing that reduces the NSP content of the meal will not only increase the protein content but also improve the overall efficacy of the product. However, the added cost of the processing will have a significant bearing on the usage of the product. Soluble NSP, particularly oligosaccharides, could have an adverse effect on digestibility. However, the response of prawns to these compounds has not been adequately studied.

Alkaloids are recognised as being anti-nutritional factors that are present in lupins grown in Australia, albeit at low concentrations. As the alkaloids in lupins provide the plant with some protection against pests, an

understanding of the tolerance of prawns to specific alkaloids in their diet would provide plant breeders with useful information for balancing the needs of protecting against pests with those of the end users.

The effect that lupin meals have on the manufacture (primarily steam pelleting) of prawn feeds and the stability of the feed pellets have not been investigated. This information would be very useful for determining whether including lupin meal in the feed will reduce processing costs and increase feed pellet stability. If this is the case, it is likely to encourage feed manufacturers to look more closely at using lupins in their feed formulations.

From the perspectives of cost, protein content and digestibility, lupin kernel meal appears to be the most appropriate lupin product for use in prawn feeds. It is essential that the factor or factors that limit the utilization of lupin kernel meal in prawn feeds be identified; whether it is a methionine limitation, oligosaccharides affecting digestibility or some other an anti-nutritional factor. The potential benefit of new cultivars or processing techniques that increase protein and reduce NSP contents of the kernel meal must be balanced against the cost of the product. The cost of the protein in these products must be compared against the cost of the protein in solvent extracted soybean meal.

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Plant ingredients in fish diets: effects of non-starch polysaccharides

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Introduction

As the world's human population continues to expand beyond 6 billion, its reliance on farmed fish as an important source of protein will also increase (Naylor et al., 2000). Most of the increase in fish production is expected to come from aquaculture, which is currently the fastest growing food production sector of the world. Aquaculture is growing at an annual rate of 9.2% compared with only 1.4% for capture fisheries and 2.8% for terrestrial meat production systems (FAO, 2003). By 2030, aquaculture will dominate fish supplies and more than half of the fish consumed is likely to originate from this sector (FAO, 2000). To sustain such a high rate of increase in aquaculture production, a matching increase in fish feed production is required. The projected total production of feeds for aquaculture in 2010 range from 25 to 32.6 million metric tonnes (IFOMA, 2000), compared to an approximate production of 13 million metric tonnes in 2000. Demand for aquaculture feeds is likely to be further increased by an increasing trend towards the intensification of omnivorous species e.g., carp in Asian countries, particularly in China.

Fishmeal is the main source of dietary protein in fish feeds. The bulk of fishmeal is used in the culture of carnivorous species e.g., salmon, trout and marine fish in western countries. Due to increased feed production, demands for fishmeal will also increase. Table 23 shows that the level of inclusion of fishmeal in the diets of all cultured species will be reduced by 2010, but increased fish production and a corresponding increase in the production of fish feeds will result in a higher demand for fishmeal. Therefore, the need to identify alternative sources of protein is vital. It is highly desirable that the selected protein sources do not conflict with human food security interests. Animal by-products, such as meat meal, blood and bone meal and poultry-by products are good protein alternatives in fish diets (Rodriguez et al., 1996; Lee et al., 2001), but health-related problems limit the use of these products in feeds for farm animals.

Table 23. Forecast of the use of fishmeal (IFOMA, 2000).

Species	% of fishmeal in the feed		X 1000 tons of fishmeal	
	2000	2010	2000	2010
Carps	5	2.5	350	516
Tilapia	7	3.5	55	60
Shrimps/Prawns	25	20	372	485
Salmon	40	30	491	569
Marine fish[1]	45	40	508	892
Trout	30	25	189	202
Catfish	3	0	15	0
Milkfish	12	5	36	28
Other marine fish[2]	55	45	127	585
Eels	50	40	173	114
Total			2316	3450

[1]Seabasses, breams, yellowtail, groupers, jacks and mullets. [2]Flatfish, cod and hake

Plant protein sources are suitable alternatives to fishmeal because being widely available, relatively cheap and safe. Research on the inclusion of plant-based ingredients in practical diets for salmonids and omnivorous fish (e.g., channel catfish, tilapia and carp) has shown that partial replacement of fishmeal is feasible (Davies et al., 1990; Webster et al., 1992). However, only a few reports have appeared on the utilisation of plant protein as the sole protein source in fish diets (Smith, 1977; Gomes et al., 1995; Kaushik et al., 1995). The presence of antinutritional factors (ANF) is a major constrain for the use of plant proteins in aquaculture diets.

Antinutritional factors

ANF are substances, which by themselves or through their metabolic products arising in living systems, interfere with food utilization, affect health and production of animals (Makkar, 1993). Table 24 summarizes ANF present in alternative fish feedstuffs. In a review on ANF (Francis et al., 2001), four groups were given:

1) factors affecting protein utilization and digestion, such as protease inhibitors, tannins, lectins
2) factors affecting mineral utilization, such as phytates, gossypol pigments, oxalates, glucosinolates
3) anti-vitamins
4) miscellaneous substances such as mycotoxins, mimosine, cyanogens, alkaloids, saponins and phytoestrogens.

ANF may also be classified according to their ability to withstand thermal processing, the most commonly employed treatment for destroying them. Heat labile factors include protease inhibitors, phytates, lectins, goitrogens and antivitamins, whereas heat stable factors are represented by saponins, non-starch polysaccharides, antigenic proteins, estrogens and some phenolic compounds (Francis et al., 2001). Although often not referred to as ANF, indigestible carbohydrates can interfere with food utilization and affect health and performance of animals. In fish diets the main source of indigestible carbohydrates originates from plant ingredients. Compared to farm animals (pigs, poultry etc.) there is relatively little knowledge on the effects of indigestible carbohydrates in fish. Oligosaccharides of the raffinose family and non-starch polysaccharides (NSP) are important constituents of a wide variety of grain legumes and cereals. In fish, their negative effects may be either due to binding to bile acids or obstructing action against action of digestive enzymes and movement of substrates in the intestine (Storebakken et al., 1998; Refstie et al., 1999).

Table 24. Antinutritional factors in alternative fish feed ingredients (Francis et al., 2001).

Plant-derived nutrient source	Antinutritional factors
Soybean meal	Protease inhibitors, lectins, phytic acid, saponins, phytooestrogens, antivitamins
Rapeseed meal	Protease inhibitors, glucosinolates, phytic acid, tannins
Lupin seed meal	Protease inhibitors, saponins, phytooestrogens, alkaloids
Pea seed meal	Protease inhibitors, lectins, tannins, cyanogens, phytic acid, saponins, antivitamins.
Sunflower oil cake	Protease inhibitors, saponins, arginase inhibitors
Cottonseed meal	Phytic acid, phytooestrogens, gossypol, antivitamins, cyclopropenoic acid
Leucaena leaf meal	Mimosine
Alfalfa leaf meal	Protease inhibitors, saponins, phytooestrogens, antivitamins,
Mustard oil cake	Glucosinolates, tannins
Sesame meal	Protease inhibitors , Phytic acid

Non-starch polysaccharides

NSP are polymers of monosaccharides joined through glycosidic linkages. There are numerous types of NSP, which differ from each other due to the sequence and composition of monosaccharides (e.g., glucose, xylose, arabinose, mannose, galactose, etc.) and the structure (the type of linkages and number, length and position

of branches). These differences result in different types of NSP, e.g., cellulose, ß-glucans, arabinoxylans, galactomannans. NSP form the major part of dietary fiber. Dietary fiber can be physiologically defined as "the dietary components resistant to degradation by mammalian enzymes" or chemically as "the sum of NSP and lignin (Bach Knudsen, 2001). NSP are generally resistant to mammalian digestive enzymes (Iji, 1999). However, NSP may be fermented by the microbial population in the intestinal lumen, resulting in volatile fatty acids which may be absorbed and used as a source of energy (De Lange, 2000). NSP do not generally exist as completely separate components in feedstuffs. In plants, NSP are predominantly present as the structural polysaccharides in plant cell walls where they are associated and/or substituted with other polysaccharides, proteins, and phenolic compounds like lignin (Bach Knudsen, 2001).

Despite the structure NSP can be classified in various ways based on their physicochemical properties: e.g., viscosity, water-holding capacity, fermentation and capacity to bind organic and inorganic molecules. Based on the reaction with water, NSP are classified as either soluble or insoluble. Soluble NSP form dispersions when mixed with water and have the ability to increase the viscosity of digesta. Insoluble NSP do not increase digesta viscosity, but can be characterized by their fecal-bulking capacity (Davidson and McDonald, 1998). The above mentioned physicochemical properties are strongly related to physiological actions of NSP when ingested by the animal (Davidson and McDonald, 1998; Chaplin, 2004). NSP are present in aquaculture feeds in a purified soluble form to stabilize the pellet (e.g. guar gum) and as an integrated part of the cell wall of plant ingredients (Table 25).

Table 25. Non-starch polysaccharide contents (g kg^{-1} dry matter) of ingredients used in aquaculture feeds (Bach Knudsen, 1997).

Ingredient	Total NSP[1]	Cellulose	Insoluble NCP[2]	Soluble NCP
Soybean meal	217	62	92	63
Rapeseed meal	220	52	123	55
Cottonseed cake	257	92	103	61
Cotton seed meal	283	90	127	66
Lupins	405	131	139	134
Sunflower cake	315	123	136	57
Peas	180	53	76	52
Alfalfa leaf meal	329	139	113	77
Wheat bran	374	72	273	29
Whole wheat	119	20	74	25
Corn gluten feed	351	75	242	34

[1]Non-starch polysaccharides [2]Non-cellulosic polysaccharides.

Viscosity

Viscosity of NSP depends on their solubility, structure (branched or linear; ferulic acid content), molecular weights, and concentration (Ellis et al., 1996; Choct, 1997; Chesson, 2001; Bach Knudsen, 2001). The high levels of viscosity associated with water soluble NSP are generated predominantly by interpenetration of individual polymer chains to form an entangled network. This process will only occur at or above a critical NSP concentration (Ellis et al., 1996). Highly viscous NSP have a low degree of branching and high ferulic acid content. High digesta viscosity delays gastric emptying and feed transit time with a resulting blood glucose lowering effect and stimulation of microbial growth in the intestine. Moreover, nutrient (e.g. fat) digestion and absorption may be compromised through less effective mixing of digestive enzymes and substrates and an increasing resistance of the unstirred water layer at the intestinal surface (Smits and Annison, 1996). This layer forms an aqueous diffusion barrier separating mixed bulk luminal contents from the brush border. Thermal methods of feed processing (e.g. extrusion) increase the release of soluble NSP, which subsequently increases the likelihood of nutritional problems due to viscosity when these diets are fed to animals (Chesson, 2001).

Recently a number of studies has been carried out by our group to assess to what extent digesta viscosity can be changed by dietary composition in different fish species (Table 26). In those studies guar gum was used as a model substrate and included at various levels. These model studies show that the effects of viscosity differ between fish species. Dietary viscosity seems to affect digesta viscosity in Nile tilapia less than African catfish and European Sea bass. Marine fish have a high drinking rate of water compared to fresh water fish. It was hypothesized that a high drinking rate of water would decrease the effects of diet on digesta viscosity. However, in contrast to this hypothesis, the experiment with European Sea bass demonstrated that the digesta viscosity in the distal part of the gut was higher at 24 ppt compared to 5 ppt. It was also found that the effect of water salinity on the effect of guar gum on digesta viscosity altered with the position in the gut. In the proximal part of the gut digesta viscosity was numerically higher at a salinity of 5 ppt versus 24 ppt (results not shown).

Table 26. Effect of guar gum inclusion on digesta viscosity in distal part of gastro-intestinal tract and apparent digestibility (ADC) of crude protein in different fish species.

Fish species	Dietary inclusion level of guar gum		
	0%	4%	8%
African catfish[1]:			
Dietary viscosity (cP)	1.0	15.7	170.9
Digesta viscosity (cP)	1.9±0.1	163.4±49.0	352.4±114.4
ADC crude protein (%)	89.5±0.77	84.1±1.03	76.8±7.08
Nile tilapia[2]:			
Dietary viscosity (cP)	0.9		42.9
Digesta viscosity (cP)	2.2±0.1		6.6±3.0
ADC crude protein (%)	91.6±0.3		84.7±3.2
European Sea bass[3]:			
Dietary viscosity (cP)	0.9	36.6	
Salinity water 5 ppt:			
Digesta viscosity (cP)	1.7±0.1	62.2±10.5	
ADC crude protein (%)	91.2±1.2	83.3±1.0	
Salinity water 24 ppt:			
Digesta viscosity (cP)	1.5±0.1	153.4	
ADC crude protein (%)	90.1±0.6	84.8±0.6	

[1]Leenhouwers et al. (Submitted). [2]Amirkolaie et al. (accepted). [3]Leenhouwers et al. (in preparation)

In line with findings in broilers, an increased digesta viscosity induced by guar gum was correlated/associated with a decline in apparent faecal digestibility (ADC) of most nutrients. This can be seen from the given ADC values in Table 26. Extrapolation of the results suggests that also regarding the digestibility Nile tilapia are less strong affected by guar gum inclusion than African catfish and European Sea bass. However, it should be realized that using guar gum as model substrate gave rather extreme high values of both dietary and digesta viscosity. Normal plant ingredients will induce much lower dietary viscosities. Therefore, to investigate the consequences of digesta/dietary viscosity in more practical fish diets a series of experiments was carried out to compare various grains which differed in viscosity (Table 27). These grains were included at a level of 40% in the diets. Both in African catfish and Tilapia, digesta viscosity differed between grains. Rye caused an increased viscosity of digesta. However, the apparent digestibilities of nutrients were not/less affected in these grain experiment (Table 27) than in the guar gum experiments (Table 26).

Table 27. Effect of different grains included at a 40% level in the diet on digesta viscosity in distal part of gastro-intestinal tract and apparent digestibility (ADC) of crude protein in different fish species.

Fish species	Grain variety at 40% inclusion level			
	Maize	Wheat	Barley	Rye
African catfish[1]:				
Grain viscosity (cP)	0.9	3.3	2.5	85.2
Dietary viscosity (cP)	1.1	1.9	1.8	7.1
Digesta viscosity (cP)	1.6±0.24	2.4±0.40	1.9±0.12	9.6±0.95
ADC crude protein (%)	79.8±0.75	80.1±2.05	80.9±0.71	77.6±1.70
Blood cholesterol (mmol/l)	4.3±0.1	4.3±0.1	4.5±0.2	4.2±0.0
Nile tilapia[2]:				
Grain viscosity (cP)	1.1	1.8	1.6	13.6
Dietary viscosity (cP)	0.9	1.3	1.3	8.6
Digesta viscosity (cP)	3.6±0.34	4.3±0.99	3.2±0.24	8.2±0.68
ADC crude protein (%)	88.5±0.9	86.5±0.3	87.6±0.7	87.2±0.8

[1]Leenhouwers et al. (in preparation). [2]Leenhouwers et al. (in preparation)

Water-holding capacity

The water-holding capacity of NSP reflects the ability to incorporate water within their matrix. Water-holding capacity is influenced by the chemical structure of the NSP, by the pH and electrolyte concentration of the surrounding fluid and by particle size (Bach Knudsen, 2001). It is important to note that the organization within the cell wall influences the water-holding capacity of NSP. For example, isolated pectin swells greatly, but when contained in the mesh of less hydrophilic substances it swells much less. Insoluble NSP have high water-holding capacities, are less well fermented, and therefore stimulate fecal bulking and shorten gut transit times (Choct, 1997; Davidson and McDonald, 1998). Soluble NSP also have high water-holding capacities and this may contribute to slow gastric emptying. However, their water-holding capacities usually diminish along the gut when they are fermented (Davidson and McDonald, 1998).

In Table 28 and 29 data are presented on the impact of diets with levels of NSP (guar gum Table 28) different sources of NSP (grains Table 29). The data on European Sea bass demonstrate that the DM content of digesta changes through the intestine, being highest in the stomach and after the stomach it declines at both the control and 4% guar gum diet. However throughout the rest of the intestine the change in DM content of digesta depends on the diet. At the NSP rich diet (4% guar gum) the DM content of digesta slightly decreases whereas at the control diet the DM content increases from the proximal part until the distal part of the intestine. Not only the NSP level in the diet, but also the type of NSP (i.e., type of grain) influences the water content of the digesta (Table 29). Both in African catfish and Nile tilapia, rye inclusion in the diet resulted in a higher water content of the digesta. From these data on DM of digesta it remains however unclear whether these effects are caused by the differences in viscosity between the diets or whether this is purely due to differences the water binding capacity between the experimental diets. These results show that dietary composition (i.e., NSP fraction) can influence the faecal characteristics. This may have practical implication in water quality management in recirculation systems. It can be hypothesized that DM content of the faeces will be related to the water stability of the faeces and thereby effect the mechanical removal efficiency from the water.

Table 28. Effect of guar gum inclusion on dry matter content (g/kg) of digesta in different parts of the gastro-intestinal tract in European Sea bass kept at two salinities (5 versus 24 ppt)[1].

Part of gastro-Intestinal tract	Dietary inclusion level of guar gum	
	0%	4%
Salinity water 5 ppt:		
Stomach	202±4.0	253±2.1
Proximal	144±13.2	89±6.3
Middle	161±6.1	69±1.2
Distal	188±8.5	65±2.1
Salinity water 24 ppt:		
Stomach	169±9.3	224±14.2
Proximal	117±0.4	82±8.9
Middle	143±1.3	79±7.9
Distal	172±9.3	74±9.5

[1]Leenhouwers et al. (in preparation).

Table 29. Effect of different grains included at a 40% level into the diet on dry matter content (g/kg) of digesta in the distal part of the gastro-intestinal tract in African catfish and Nile tilapia.

Fish species	Grain variety at 40% inclusion level			
	Maize	Wheat	Barley	Rye
African catfish[1]:	197±9.0	201±8.5	227±1.2	170±2.6
Nile tilapia[2]:	135±11.0	102±11.5	130±4.0	100±8.7

[1]Leenhouwers et al. (in preparation). [2]Leenhouwers et al. (in preparation)

Fermentability

Intestinal bacteria, particularly those present in the hindgut, can ferment NSP to obtain energy for their own growth and maintenance. Volatile fatty acids (VFA) such as acetate, propionate and butyrate and the gases H2, CH4 and CO2 are the major end products of fermentation (Williams et al., 2001). Volatile fatty acids can be rapidly absorbed by the intestine and thus may contribute to the energy requirements of the animal. Volatile fatty acids have been found in the gut and/or blood of many herbivorous fish species (Rimmer and Wiebe, 1987; Titus and Ahearn, 1988; Clements et al., 1994; Kandel et al., 1994; Clements and Choat, 1995) and omnivorous species (Smith et al., 1996). Concentrations of acetate in fish are much lower (15-39 mmol.l-1) than in found in ruminants (60-120 mmol.l-1) or terrestrial hindgut fermenters such as the horse, rabbits and humans (approx 100 mmol.l-1), but are comparable to those found in other ectotherms like the iguana (16 mmol.l-1) and the tortoise (23 mmol.l-1) (Clements et al., 1994). Several studies have suggested that fermentation may contribute to energy metabolism in herbivorous fish species, such as tilapia and carp. For carnivorous fish species such as salmon and sea bass, the existence of a microflora capable of fermentation seems less likely. The evolutionary diets of all carnivorous fish vary in protein content, but are consistent in being low in carbohydrate. Therefore, carnivorous fish apparently never developed the capacities to adaptively modulate digestive characteristics in response to changes in diet composition. This limitation restricts the ability of carnivorous fish to effectively utilize lower cost diets formulated with higher levels of carbohydrates (Buddington et al., 1997).

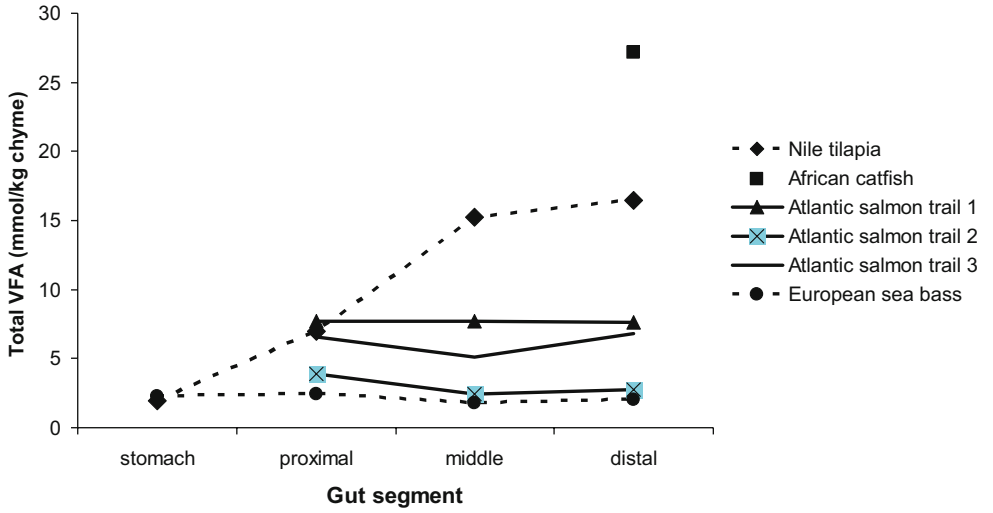

Figure 12. Total volatile fatty acid (VFA) concentration in chyme in different parts of the gastro intestinal tract of different fish species. Values are averages over dietary treatments, because dietary composition (NSP) had no effect (Leenhouwers et al., in preparation).

The occurrence of fermentation as affected by dietary NSP content was studied in various experiments performed in our group. Fermentation was quantified by measuring the amount of different volatile fatty acids (VFA) in the chyme of the fish. In line with literature, levels of total VFA were considerably lower than in farm animals (cattle, pigs and poultry; ranging from 60 to120 mmol/kg) (Figure 12). In most experiments, total VFA content was not influenced by the applied dietary treatments (differences in NSP content). In the more carnivorous fishes (salmon and sea bass) the level of VFA remained constant throughout the gastro intestinal tract and was lower than in Nile tilapia and African catfish. In Nile tilapia (herbivorous) the total VFA concentration increased from stomach until the distal part of the intestine (Figure 12). In all fish species, the major part of VFA produced was acetic acid (>80%), which is much higher than the acetic acid contribution in chyme of pigs and poultry.

Capacity to bind organic and inorganic molecules

Some NSP, like pectins, have an influence on cation adsorption, particularly calcium, zinc and iron. The mineral cation-binding capacity of NSP-rich foods and cereals in particular, is largely due to the presence of phytates (Davidson and McDonald, 1998). The phosphate groups of phytate that are often associated with NSP are negatively charged at intestinal pH, thereby being capable of reacting with positively charged minerals. In Figure 13 data on crude ash digestibility in Nile tilapia suggest that also in fish the NSP content can influence the mineral absorption. In Nile tilapia, inclusion of guar gum resulted in a decreased ash digestibility, whereas cellulose had no effect. Whether such effects are present is other fish species requires further research.

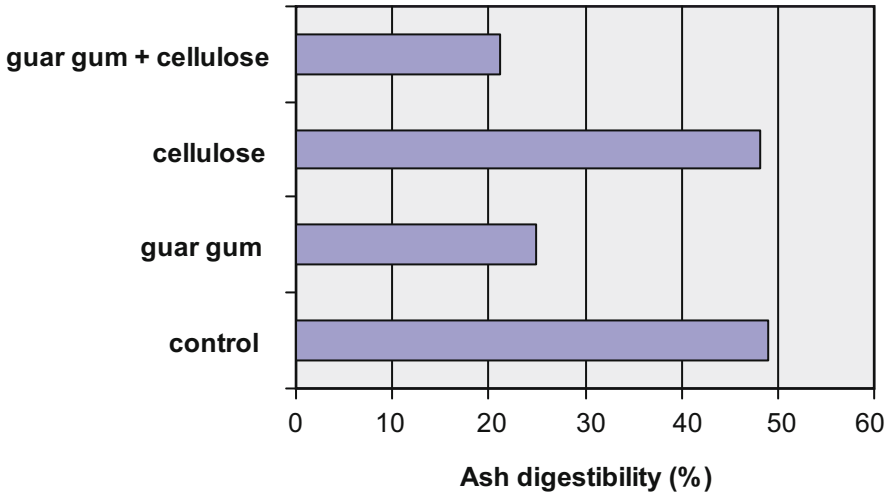

Figure 13. Effect of type of NSP (cellulose vs guar gum) on crude ash digestibility in Nile tilapia. Cellulose was included at a level of 0 or 8% in the diet and guar gum also at a level of 0 or 8% (Amirkolaie et al., accepted).

The binding of bile acids by soluble or insoluble NSP may significantly impact lipid absorption and cholesterol metabolism. Adsorption of bile acids by NSP is one of the proposed mechanisms for the hypocholesteremic effect of NSP. Increased fecal excretion of bile acids leads to the increased metabolism of cholesterol in the liver, thus lowering serum cholesterol levels (Chaplin, 2004). In African catfish, blood cholesterol levels were not different between different grains (maize, wheat, barley versus rye) at an inclusion level of 40% in the diet (Table 27). This suggests that in fish such on effect of NSP on blood cholesterol level is absent. However, further research in more fish species is needed to validate this hypothesis.

Concluding remarks

In future the content of non-starch polysaccharides (NSP) in commercial fish diets will increase as a consequence of the increase in the inclusion levels of plant ingredients into these diets. Currently there is relatively little information on the effects of increased dietary NSP levels for fish. Preliminary data suggest that NSP can affect digesta viscosity in fish and that the influence of NSP differs between fish species. The extent of fermentation of NSP in the intestine of fish requires further research. Similarly from the current knowledge it is not fully clear whether NSP content in various fish species have large consequences for the digestibility and/or availability of important nutrients.

Acknowledgements

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Implications of lupins on aquaculture feed extrusion processing

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Introduction

Extrusion technology is used to produce most modern fish feeds, and is a process where the ingredients are blended, moistened and then cooked whilst under pressure. Extruded pellets generally posses improved binding properties as well as an increased porosity compared to the traditional steam/compressed pellets. In high-energy salmonid feeds this increased porosity is useful in post extrusion oil application, allowing higher oil levels to be achieved under both passive and vacuum infusion processes. The increased binding strength of the pellet is also a benefit, with reduced fines and increased durability when compared to traditional compression pellet.

Extrusion of lupin kernel meals and protein concentrates

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, durability, oil and water absorption. Preliminary studies have investigated the processing of lupins in diets for fin-fish and salmonids using extrusion technology (Evans 1999; Glencross and Sipsas, 2004). 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.

Table 30. Formulations for extruded diets used in Atlantic salmon experiments.

Ingredient	Reference	LKM	LPC	BKM	MKM	MPC	SBM
Yttrium Oxide	0.10	0.07	0.07	0.07	0.07	0.07	0.07
Pre-mix vitamins	0.50	0.35	0.35	0.35	0.35	0.35	0.35
Fish oil	15.00	10.50	10.50	10.50	10.50	10.50	10.50
Wheat flour	14.40	10.08	10.08	10.08	10.08	10.08	10.08
Fish meal	70.00	49.00	49.00	49.00	49.00	49.00	49.00
L. luteus (cv. Wodjil) KM		30.00					
L. luteus PC			30.00				
L. angustifolius (cv. Belara) KM				30.00			
L. angustifolius (cv. Myallie) KM					30.00		
L. angustifolius (cv. Myallie) PC						30.00	
Soybean meal							30.00

In 2004 a series of diets were made, using a Wenger X185 mini-extruder at the Australasian Experimental Stockfeed Extrusion Centre at Roseworthy College in South Australia, for the Atlantic salmon components of the program. There were seven diets (Table 30) made with the reference/basal diet comprising principally of fishmeal, wheat and fish oil. The test diets were based on a 70:30 ratio of reference diet to test ingredient allowing the uniform examination of the effects of each ingredient on pellet quality. Oil was applied post extrusion by vacuum infusion, as high oil content in the extruder provides too much lubrication, diminishing the pressure and force generated, so diminishing the expansion of the pellet. Approximately 120 – 150 kg of each diet was made sending 50 kg of each to Norway and Tasmania for the Atlantic salmon projects and the remainder was sent to Western Australia for storage. Samples of each diet mash and the pellets were also kept for further assessments.

Viscosity assessment and variability

Rapid Viscosity Analysis (RVA) is a technique used to assess the rheology of a sample. It measures the resistance to a small rotor inside a cup that contains the sample. The moisture content and thermal management of this cup can be varied to mimic conditions that occur during extrusion. Typically a thermal regime used is; 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 15). Ideally the RVA data need to be correlated with extrusion data to provide meaningful interpretation but relative hydration responses and peak viscosities can provide an indication of prospective extrusion energy and water demands.

To understand the variability in viscosity parameters amongst *L. angustifolius,* 15 cultivars sourced from Wongan Hills Research Station (2003 season) were analysed for their viscosity using Standard 1 heating profile (Figure 14). Substantial variability was noted among the different cultivars, the presence and absence of certain peaks and also the magnitude of those peaks (Figure 15). Figure 15 shows only a subset of those 15 cultivars. Variability was noted not only among cultivars but also from different regions/seasons.

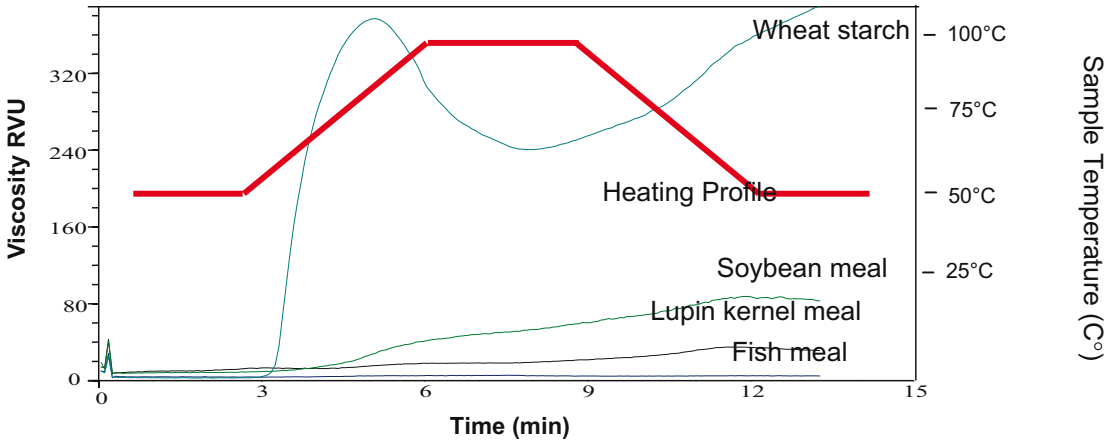

Figure 14. RVA profile of wheat starch, soybean meal, lupin kernel meal and fish meal on a standard heat-hold-cool program.

Diet mash viscosity

In addition to assessment of the individual ingredient samples, evaluation of the diet mashes (as extruded) was also undertaken allowing the examination of RVA as a predictive tool of extruded pellet features. On examination it was noted that the viscosity of samples is not necessarily additive. Notably, some ingredients that showed high individual viscosities resulted in diets with relatively low mash/diet viscosities. In contrast, some ingredients (e.g. *L. angustifolius* kernel meals) with relatively low individual viscosities resulted in relatively high mash/diet viscosities.

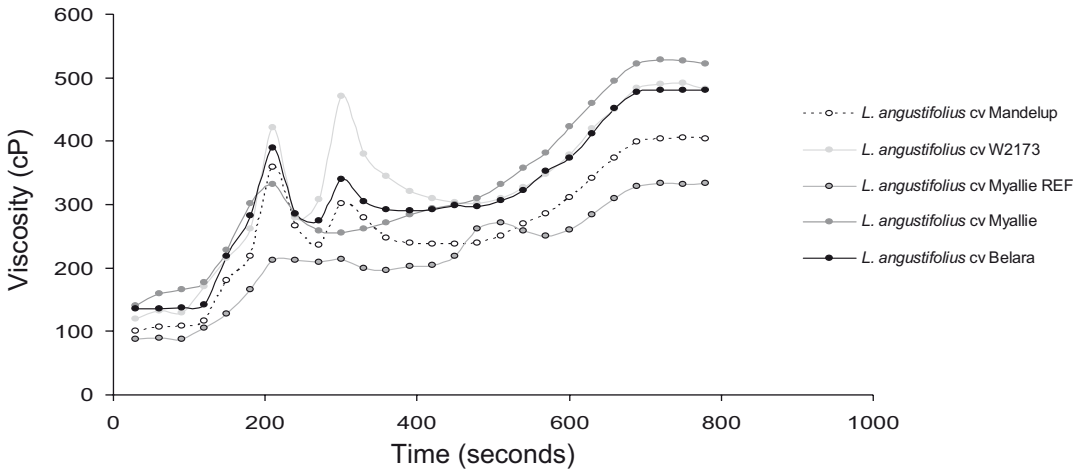

Figure 15. Variability in viscosity among *L. angustifolius* kernel meals.

Pellet durability

One of the most useful assessments of durability being regularly used by the aquaculture feeds industry is the Durability On a Realistic Scale (DORIS) technique. This technique uses a special piece of equipment the 'DORIS'. A weighed amount of pellets are passed through the instrument, collected and then sieved and the relative proportions of the original weight resulting in the various fractions determined. In the present example duplicate samples of the Atlantic salmon experiment diets were assessed using the DORIS and their durability parameters determined (Table 31).

Table 31. Extruded pellet durability as assessed using DORIS.

	<1180µm	<3000µm	>3000µm
Myallie Protein Conc.	1.5%	5.2%	93.2%
Soybean Meal	2.8%	3.8%	93.4%
Luteus Protein Conc.	1.8%	2.7%	95.5%
Fishmeal Reference	0.9%	1.5%	97.7%
Belara Kernel Meal	0.2%	0.2%	99.6%
Myallie Kernel Meal	0.2%	0.0%	99.8%
L. luteus Kernel Meal	0.1%	0.0%	99.9%

Using RVA to predict pellet durability

Using the data collected from the extruded diets, the RVA assessment of the extrusion mashes and the durability data from the DORIS assessment, multiple regression modelling was conducted to determine which part of the RVA assessment provided the best indication of the pellet durability. Figure 16 shows the regression value for each 30 second time point during the RVA assessment. It is apparent that there is an increasing trend in the strength of the RVA viscosity level and the pellet durability. At the end of the RVA assessment the R^2 values were consistently over 90%, indicating that the final viscosity provides the best RVA-based prediction of potential pellet durability.

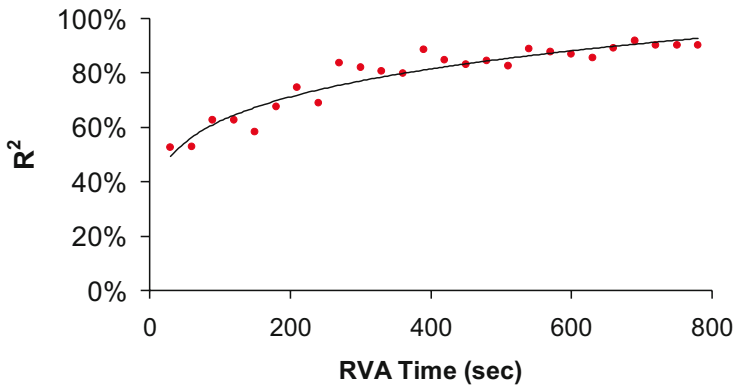

Figure 16. Regression of each 30 second time point of the RVA viscosity of diet mashes and DORIS durability of the extruded pellets produced from those mashes.

Processing throughput and Kernel Hardness

Discussions with the aquaculture feed industry indicated that milling lupin kernel products through their hammermills substantially increased their throughput time and increased their energy costs. Figures of a 20% reduction in milling throughput were touted. In response to this a preliminary assessment of the variability in lupin kernel hardness was undertaken to determine what level of variability was present among the different species and cultivars.

The hardness of the kernels/cotyledons of different lupin species and different cultivars of *L. angustifolius* was assessed using a TA.XT2*i* texture analyser. This machine measures the force applied by a knife blade as it passes through the kernel when applied at a constant rate (0.1 mm/sec).

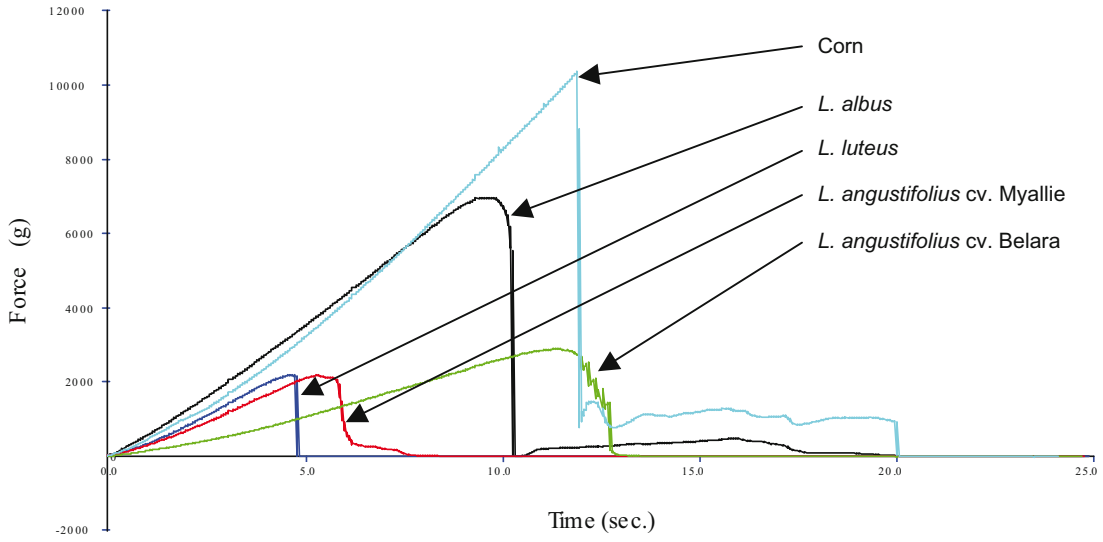

Figure 17. TA.XT2*i* texture analyzer response to various *L. angustifolius* cultivars, *L. luteus* cv Wodjil and *L. albus* cv Kiev mutant, and corn kernels.

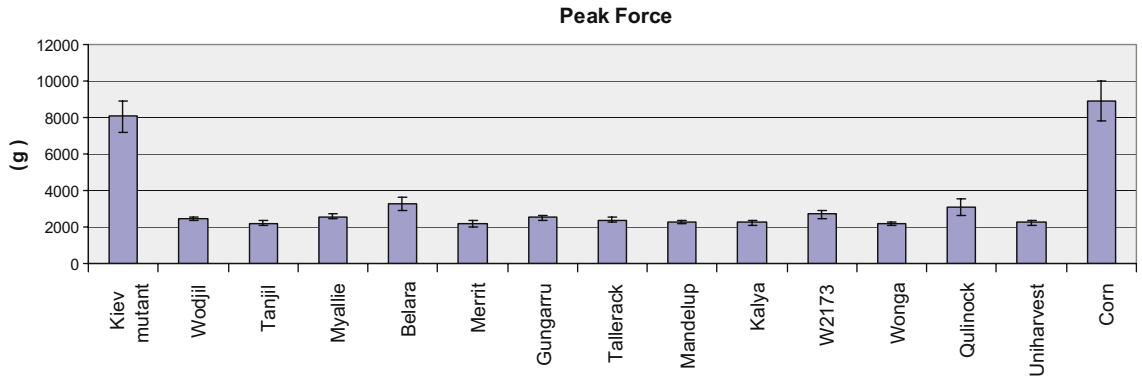

Figure 18. TA.XT2*i* texture analyzer peak force required to cleave various *L. angustifolius* cultivars, *L. luteus* cv Wodjil and *L. albus* cv Kiev mutant kernels.

The data from the TA.XT2*i* texture analyser meter provides an indication of the level of variability in hardness among the different grain. However, direct interpretation of milling outcomes from this is not so straightforward. Further assessment on grain hardness is required to achieve a better idea of how the TA.XT2*i* texture analyser data can be applied to a measure of 'milling' hardness that is relevant to industry.

Future for functionality assessment

Further assessment is being made of the variability among *L. angustifolius* kernel meals on the pellet extruding process. These studies will also examine the RVA and DORIS assessments to further add to our knowledge of how lupins influence processing of aquaculture feeds.

Additional studies on energy demands for certain throughput of lupin kernels will be sought, as will additional assessments of hardness index. This information will then be used to inform the aquaculture feed industry about milling issues associated with these products.

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Glencross, B.D. (Ed) 2005. *Proceedings of the second workshop for Seeding a Future for Grains in Aquaculture Feeds - 26 May 2004*, Fisheries Occasional Publications No. 23, Department of Fisheries, Western Australia, 51p.

Appendix 1 – Program 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.
- Glencross, B., Evans, D., Dods, K., McCafferty, P., Hawkins, W., Maas, R., Sipsas, S. (2005) Evaluation of the digestible value of lupin and soybean protein concentrates and isolates when fed to rainbow trout, *Oncorhynchus mykiss*, using either stripping or settlement faecal collection methods. Aquaculture 245, 211-220.

Appendix 2 – Other Relevant Publications

- Glencross, B., Curnow, J., Hawkins, W., Kissil, G.WM., Peterson, D. (2003) Evaluation of the feed value of a transgenic strain of the narrow-leaf lupin (*Lupinus angustifolius*) in the diet of the marine fish, *Pagrus auratus.* Aquaculture Nutrition 9, 197-206.
- Glencross, B.D., Boujard, T., Kaushik, S.J. (2003) Influence of oligosaccharides on the digestibility of lupin meals when fed to rainbow trout, *Oncorhynchus mykiss.* Aquaculture 219, 703-713.
- Glencross, B., Hawkins, W., Curnow, J. (2004) Nutritional assessment of Australian canola meals. I. Evaluation of canola oil extraction method and meal processing conditions on the digestible value of canola meals fed to the red seabream (*Pagrus auratus,* Paulin). Aquaculture Research 35, 15-24.
- Glencross, B., Hawkins, W., Curnow, J. (2004) Nutritional assessment of Australian canola meals. II. Evaluation of the influence of canola oil extraction method on the protein value of canola meals fed to the red seabream (*Pagrus auratus,* Paulin). Aquaculture Research 35, 25-34.