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The development of grain-use in aquafeeds in Australia

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Introduction

Aquaculture is the fastest growing food-producing sector in the world, growing at 8.8% annually since 1950 and far out-performing other livestock food sectors, which have grown by only 1% for beef and veal, between 2 and 3% for lamb, mutton and pig meats and 4.9% for chicken meat (Tacon, 2004; FAO, 2006b). In 2004, aquaculture production was 59.4 million tonnes (mt) worth US$70 billion and accounted for almost 50% of global aquatic food consumption (FAO, 2006b) (Figure 1). Because landings of capture fisheries have plateaued at 80 to 90 mt per year since the 1980's, and are expected not to increase in the foreseeable future, aquaculture production must continue to grow and attain an annual production of at least 100 mt by 2050 just to maintain the current per capita consumption of 16 kg. While this represents an enormous challenge for the aquaculture industry, both in terms of environmental sustainability, resource use and social responsibility, none will be more important than finding sufficient feed to grow these aquaculture animals profitably.

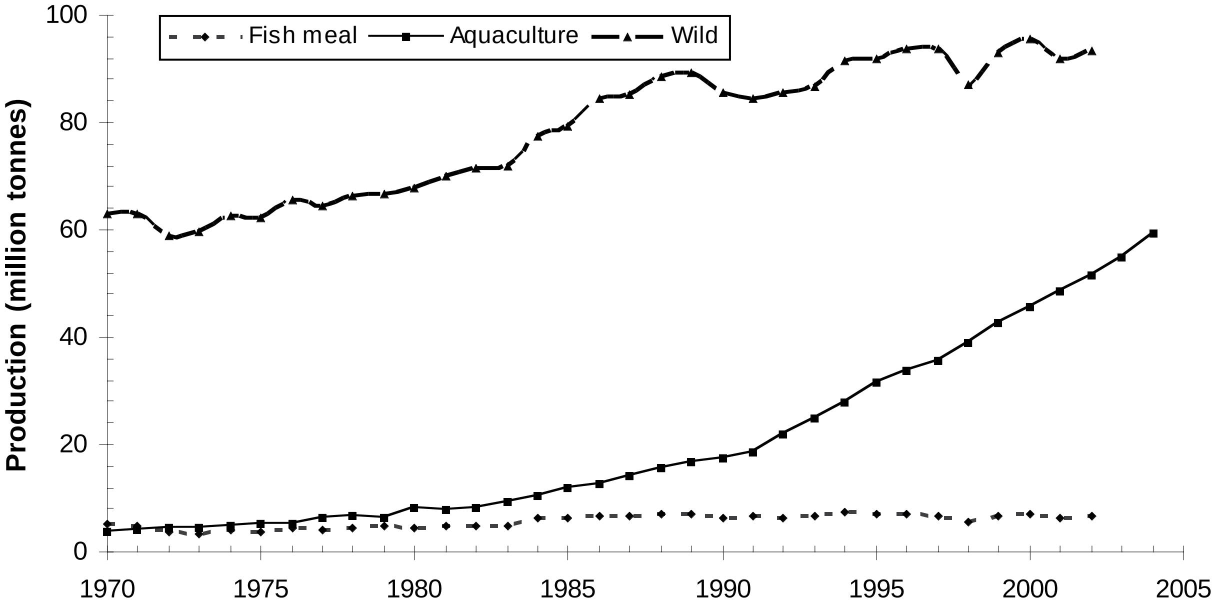

Figure 1. Total annual production of fish meal, aquaculture (including aquatic plants) and wild (capture) fisheries 1970-2004 (data from FAO, 2006a,b and IFFO, 2006).

The global expansion and intensification of aquaculture has brought with it an increasing requirement for compounded feeds. Aquatic plants and filter-feeders (predominantly carp species and mollusks) make-up about half of total aquaculture production and thus feeding them is less of a problem. However, the other 50% of production comes from animals that consume industrially compounded aquafeeds or are fed directly on low-value ('trash') fish. It is estimated that almost 20 mt of compounded aquafeeds were consumed by aquaculture in 2004 and this is expected to increase to 25 mt by 2010 (FAO, 2006b). A particular concern in servicing this demand is that compounded aquafeeds are major consumers of fish meal and fish oil, but since 1985 production of these commodities has stabilized at 6-7 mt and 1 mt, respectively (Shepherd et al., 2005; IFFO, 2006) (Figure 1). It was estimated that the 19.5 mt of compounded aquafeed needed for aquaculture in 2003 used about 2.7 mt of fish meal and 0.7 mt of fish oil (Tacon, 2004; FAO, 2006b). This represents 42 and 70% of total world production of these commodities, respectively. If aquaculture production increases as expected and usage of fish meal and fish oil was to continue at current levels, by 2020 aquafeeds would exhaust the total global supply of fish oil and consume more than 60% of this global fish meal production. This is an unlikely scenario. As demand for fish oil and fish meal increase, the price of these commodities will also increase to a point where it will no longer be economically viable for aquafeed manufacturers to sustain these usage rates. This highlights the need for research to reduce aquaculture's reliance on fish meal and fish oil as major ingredients in compounded aquafeeds.

Australia as a player in global aquaculture

For the 2003/04 statistical year, Australia's total aquaculture food-production was 52 thousand tonnes (kt) worth US$ 526 million (m) – literally, a drop in the ocean in a global context (O'Sullivan et al., 2006). Making up more than 90% of volume and 85% of value of production, the top six cultured 'species' were southern bluefin tuna (9.3 kt; $151 m), salmonids (Atlantic salmon and trout) (17.2 kt; $131 m), edible oysters (13.6 kt; $76 m), prawns (3.8 kt; $58 m), barramundi (2.8 kt; $24 m) and native freshwater fish (predominantly silver perch) (0.4 kt; $7 m). Similar to what is occurring globally, Australian aquaculture production has increased by an average of 10-12% annually between 1970 and 1998 but since 2000 this rate has declined to less than 10% by volume and 3% by value. With a long coastline of about 37,000 km, a fishing (economic) exclusion zone (EEZ) of 8.9 million square kilometers and considerable inland fresh and saline waters, Australia has the water resources to be a significant aquaculture producer. However, environmental, conservation and social issues will restrict Australia's aquaculture industry to a small size and one most likely primarily satisfying domestic aquatic food needs.

The Australian aquafeed industry is also quite small with current annual production estimated to be no more than 50 kt. Salmonids are the main users of this feed since southern bluefin tuna are fed almost entirely on fresh pilchards. While Australia may never be a significant aquaculture-producing country, its large broad-acre production of food crops and proximity to aquaculture producers in SE Asia and China mean that it is well positioned to be a major supplier of feed ingredients and/or manufactured aquafeeds to the global market place. More than 90% of global aquaculture production comes from food-improvished Asia, with China alone accounting for 74% of total world production (FAO, 2006b). Additionally, Australia has a strong aquaculture R&D skill base and good aquaculture nutrition research infrastructure that has already shown, and continues to demonstrate, the cost benefit of including significant amounts of Australian feed ingredients in aquafeeds.

Collaborative Australian aquaculture nutrition research

The need to develop cost-effective aquafeeds with a low reliance on marine feedstocks was recognized early in Australia with the establishment of the Fish Meal Replacement (FMR) subprogram in 1993. This was a nationally-coordinated program funded by the Australian Fisheries Research & Development Corporation (FRDC) with additional funds from other R&D Corporations, private industry and collaborating scientific institutions. This program was led by was Dr Geoff Allan, NSW Department of Primary Industries and Fisheries with research being carried out as individual species projects. In 1996, the FMR was continued for another three years as the .Aquaculture Diet Development (ADD) subprogram, again under the leadership of Dr Geoff Allan but with projects now aligned on a research discipline basis across species. In this FMR/ADD work, aquaculture nutrition scientists from eight state and federal research organizations and universities implemented a structured and collaborative approach to the problem – a world first for Australian aquaculture. Four 'template' species were selected for investigation: (i) a cold-water diadromous carnivorous fish (Atlantic salmon, *Salmo salar)*; (ii) a marine, predominantly carnivorous prawn (giant tiger prawn, *Penaeus monodon*); (iii) a warm-water euryhaline carnivorous fish (barramundi, *Lates calcarifer*); and (iv) a freshwater, predominantly herbivorous fish (silver perch, *Bidyanus bidyanus*). These species were chosen because of their importance for development of Australian aquaculture and because they best represented the feeding habit of species that make up most of global aquaculture production for which compounded aquafeeds are the main source of food (Table 1).

Table 1. Production, feeding habit and dietary protein and lipid specifications of globally-important and Australian aquaculture species that are fed compounded aquafeeds during grow-out.

Species	2004 Global production (kt)[1]	Habit FW = freshwater B = Brackish M = Marine	Diet specifications (% as-fed basis)[2]	
			Protein	Lipid
Grass carp *Ctenopharyngodon idella*	3,877.0	FW herbivore	30-35	4-8
Common carp *Cyprinus carpio*	3,388.0	FW omni-herbivore	31-38	8-10
Tilapia *Oreochromis* spp.	1,772.0	FW/B omni-herbivore	30-35	10-12
Atlantic salmon *Salmo salar*	1,245.0	M carnivore	40-45	30-35
White shrimp/prawn *Metapenaeus* & *Penaeus*	1,503.0	M/B omnivore	32-36	6-8
Tiger shrimp/prawn *Penaeus* spp.	769.0	M/B omni-carnivore	40-45	8-10
Milkfish *Chanos chanos*	574.0	M/B Plank-herbivore	38-42	7-10
Rainbow trout *Oncorhynchus mykiss*	505.0	FW carnivore	40-45	18-22
Sea bream & kingfish *Sparidae* & *Carangidae*	391.0	M carnivore	45-50	12-15
Channel catfish *Ictalurus punctatus*	353.0	FW omni-herbivore	26-32	4-6
Other catfish *Clarias* & *Pangasius*	308.0	FW carnivore	32-35	6-8
Barramundi *Lates calcarifer*	30.0	M/FW carnivore	45-50	12-15
Silver perch *Bidyanus bidyanus*	0.3	FW herbivore	33-35	5-8

[1] FAO (2006a).

[2] Collated data from Guillaume et al. (2001) and literature compiled by the author.

It was always intended that the research done in the FMR/ADD should have application to global aquaculture and that Australia's benefit may be greatest as a supplier of terrestrial feedstocks to the global aquafeed market. The approach with each species was four-fold: (i) develop sound research methods to investigate the potential of Australian feedstocks in aquafeeds; (ii) determine the animal's acceptance of alternative (terrestrial) feedstocks; (iii) determine the digestibility of feed ingredients that had the greatest potential to replace fish meal in aquafeeds; and (iv) increase knowledge about the nutritional requirements of the targeted species. This accumulated knowledge was subsequently applied to develop least-cost aquafeeds in which fish meal was partially or completely replaced by terrestrial feedstocks. In collaboration with aquaculture farmers and aquafeed manufacturers, the developed new aquafeeds were tested and the laboratory findings validated under on-farm conditions. Strong linkages were also formed between the FMR/ADD and the closely aligned nutritional projects in the Aquaculture Cooperative Research Centre. The FMR/ADD was succeeded by the FRDC Aquaculture Nutrition subprogram which continues to this day under the management of Dr Robert van Barneveld, Barneveld Nutrition P/L. A summary of the main research findings coming from the FMR/ADD is outlined in the following sections. The more recent work (since 1999) will be discussed by others at the symposium.

Identifying alternative feed ingredients for use in aquafeeds

The suitability and extent to which feed ingredients can be substituted for one another in any animal diet is determined by several biotic and non-biotic factors:

(i) Whether the animal will readily eat the diet containing the ingredient under consideration. This can be a complex issue as acceptance of the ingredient almost invariably will be affected by its inclusion level, decreasing as inclusion level in the diet increases. Other more palatable ingredients may be used in the formulation in an attempt to overcome or mask the unpalatability of the disliked ingredient.

(ii) Whether the intended feed ingredient contains some anti-nutritional factor/s or is contaminated by an injurious substance. Restricting the inclusion level of the feed ingredient in the diet may still permit its use without undue harm to the animal or end-consumer.

(iii) Whether the ingredient is readily digested by the animal and in a manner that does not compromise the animal's metabolism. For example, many strict carnivorous fish can readily digest starch and other types of simple carbohydrates but may not be able to utilize the absorbed sugars at a rate sufficient to prevent an insulin-deficient like metabolic response in the animal.

(iv) Whether the chemical composition of the intended ingredient limits or prevents its inclusion because of a formulation incompatibility. For example crustaceans have an intolerance to diets containing more than about 10-12% total lipid while many warm-water carnivorous fish grow best on diets containing at least 50% protein (Table 1). Thus ingredients containing high amounts of lipid such as most meat meals have little place in a prawn diet while for most carnivorous fish, low protein meals such as chick pea are similarly unable to be 'physically' accommodated in a high protein formulation.

(v) Whether including the intended ingredient induces undesirable physical properties in the manufactured (pelleted) aquafeed. Pelleted aquafeeds have to be durable for transport and storage and have high water stability when fed-out. The physico-chemical properties of ingredients singularly and collectively can interact to have positive or negative effects on pellet manufacture (and cost). Moreover, these effects can vary depending on the type of manufacturing process used.

(vi) Whether it is economic to include the intended ingredient in the diet. In addition to the obvious comparative cost of the digestible nutrients contributed by the ingredient, other economic considerations have to be considered: reliability, seasonality and quantity of supply, need/cost for additional storage equipment (silo or sheds etc) and marketing consequences of including the ingredient (consumer attitudes to BSE, end-product colour and taste etc).

The above considerations are essentially the same whether formulating diets for terrestrial or aquatic animals. The main differences are species-specific issues – acceptability and digestibility of the ingredient and the extent to which the ingredient can be accommodated in the formulation while meeting prescribed nutritional specifications.

Digestibility of Australian-sourced feed ingredients

In the FMR/ADD, about 60 Australian-sourced feed ingredients were selected as potential ingredients for inclusion in aquafeeds. Table 2 lists the major groups of feed ingredients that were examined and the typical Australian production and export volumes. Volume of production, and particularly the quantities available for export, were important factors when deciding which feed ingredients had the greatest potential for use in aquafeeds. The ingredients were first assessed with respect to their nutrient (and energy) composition and apparent digestibility as determined with silver perch. This provided a quick screening procedure for selecting ingredients for further evaluation with each of the target species.

Table 2. Rounded average annual production and export of major broad-acre crops and abattoir byproduct meals in Australia 1996-2005[1].

Product	Total produced (kt)	Export (kt)
Coarse grains		
Barley	6,686.0	4,707.0
Maize	366.0	34.0
Rice (paddy)	1,033.0	469.0
Sorghum	1,884.0	422.0
Wheat	21,324.0	15,269.0
Pulses		
Chick pea	183.0	185.0
Field pea	392.0	250.0
Lupin (*L.angustifolius*)	1,287.0	713.0
Mung bean	49.0	?
Oilseeds		
Canola	1,684.0	158.0
Cottonseed	888.0	385.0
Soybean	67.0	5.0
Sunflower	97.0	7.0
Plant protein meals		
Canola	228.0	0.3
Cottonseed (d/h)	160.0	25[2]
Lupin (d/h)	?	?
Soybean	32.0	3.0
Sunflower	48.0	?[2]
Wheat gluten	?	?
Land animal meals		
Meat & bone meal	517.0	}217.0
Blood meal	31.0	
Poultry meal	46.0	

[1] Data compiled from ABARE (2006) for plant products and from Anon (2002) for land animal meals.

[2] Includes sunflower meal.

Ingredients that were in relatively low supply or whose nutritional composition and/or apparent digestibility was poor or appeared to limit their usefulness as fish meal replacements in particular species, were not examined further.

Table 3 compares the apparent digestibility of feed ingredients that were determined with silver perch, prawn, barramundi and Atlantic salmon. The absence of data for some species reflects the perceived unsuitability of the ingredient for that species. Some general observations can be made:

(i) Protein digestibility was high for both plant and animal ingredients and fairly similar for all four species. The protein digestibility of dehulled narrow-leaf lupin (*Lupinus angustifolius*) and wheat gluten was exceptionally high and near enough to 100% for all species.

Table 3. Typical crude protein (CP) and gross energy (GE) content (dry matter basis) and apparent digestibility coefficients[1] for selected feed ingredients determined with silver perch, prawn (P. monodon), barramundi and Atlantic salmon.

Ingredient	Composition		Apparent digestibility coefficient (%)							
	CP	GE	Silver perch		Prawn		Barramundi		Atlantic salmon	
	(%)	(kJ/g)	CP	GE	CP	GE	CP	GE	CP	GE
Coarse grains										
Sorghum	14.5	18.8	78	38	–	–	–	–	–	–
Wheat (ASW)	12.2	18.3	100	53	–	–	–	–	–	–
Pulses									–	–
Chick pea	20.8	19.4	82	55	–	–	–	–	–	–
Field pea (Dunn)	25.5	17.0	81	51	89	83	–	–	–	–
Lupin (narrow leaf)	34.1	17.9	97	51	88	45	–	–	–	–
Lupin (white)	37.6	20.9	96	70	–	–	–	–	–	–
Plant protein meals										
Canola (solvent)	36.6	19.9	83	58	79	53	81	56	81	63
Cottonseed (d/h)	48.1	19.9	83	53	–	–	–	–	–	–
Lupin (d/h narrow)	43.6	20.7	100	70	94	68	98	62	–	–
Maize gluten	62.0	24.1	95	105	–	–	–	–	–	–
Peanut	41.2	19.7	98	77	–	–	92	69	–	–
Soybean (full-fat)	35.8	23.3	92	80	–	–	85	76	–	–
Soybean (solvent)	47.8	17.0	95	78	92	71	86	69	93	75
Wheat gluten	76.9	23.1	100	100	100	100	102	99	100	90
Land animal meals										
Blood meal (ring)	94.9	23.9	90	100	–	–	–	–		
MBM (beef)	49.2	16.1	72	75	77	61	54	58	–	–
MBM (lamb)	54.3	16.2	74	81	74	55	64	67	–	–
MBM (mixed)	55.9	16.8	83	85	–	–	–	–	–	–
Poultry offal meal	60.3	22.7	85	94	–		79	77	–	–
Marine protein meals										
Fish (Tasmanian)	73.2	21.3	92	90	93	89	–	–	90	89
Fish (Danish)	72.9	21.5	94	98	–	–	88	83	–	–
Fish (Anchovy)	70.2	20.9	89	89	–	–	–	–	–	–

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[1] Data from: Allan et al. (2000) and Booth et al. (2001) for silver perch; Smith et al. (2001) for prawn; Boonyaratpalin and Williams (2002) for barramundi; and Carter (1999) for Atlantic salmon.

(ii) Energy digestibility was generally much lower than the protein digestibility of the same feed ingredient and higher values were generally observed for silver perch than for the other more carnivorous species. The energy digestibility of wheat gluten was virtually 100% for all species, which follows from the high content and digestibility of the protein in gluten, but that of dehulled lupin was only mid-range and around 60-70%.

Australian feed ingredients with high aquafeed potential

The high dietary protein and lipid specifications required for Atlantic salmon feeds (Table 1) severely restricts the feed ingredients that can be used to replace fish meal in these diets (Carter and Hauler, 2000). Moreover, the European Union ban on using land animal protein (LAP) in feeds for food animals marketed in Europe, a major destination for Australian-produced Atlantic salmon, effectively eliminates these ingredients from aquafeeds for this species. This ban is under review and some lessening of use for aquafeeds is expected, particularly for LAP produced in countries such as Australia and New Zealand, which are BSE-free. Abattoir by-product meals are well accepted by both herbivorous and carnivorous fish and crustaceans and have been successfully used to replace up to two-thirds (prawn) and up to100% (silver perch and barramundi) of the protein of fish meal without adverse effects on animal productivity or consumer eating preference (Stone et al., 2000; Smith et al., 2001; Williams et al., 2003a,b; Allan and Rowland, 2005). Thus, the extent to which abattoir meals can be used as fish meal replacements will be governed by the comparative cost of alternative meals to supply digestible nutrients (predominantly protein) and energy and marketing restrictions that apply to the use of LAP in feeds for food fish.

The suitability of plant meals as dietary fish meal substitutes is very much dependent on the aquaculture species to be fed. Silver perch are physiologically well adapted to accept and efficiently utilize plant materials and even low-protein feed ingredients such as the pulse grains can be used at comparatively high inclusion rates since the dietary protein and energy specifications are also low for this fish (Table 1). Allan and colleagues have demonstrated the cost-effectiveness of using a large variety of feed ingredients in diets for silver perch (Allan et al., 2000; Booth and Allan, 2003). For more carnivorous species, their generally higher dietary protein specification and lower tolerance to high dietary carbohydrate intake, restricts the choice of suitable plant protein meals. Gluten meals (either of wheat or maize origin) have been found to be very well accepted and to have high digestibility for all four species examined in the FMR/ADD. However, their use as a major replacement of fish meal in aquafeeds is constrained by their high comparative cost, very low lysine content and potentially undesirable pelleting properties, which at inclusion levels above about 10% can result in pelleted feed that is too hard to be accepted by the fish. Devitalizing the gluten prior to its use in feed pellet manufacture will overcome the latter problem but add further to its cost. At low inclusion rates of 5-6%, vital gluten is a very useful pellet binder and is widely used in prawn diets for this purpose. Of the other plant protein meals, research efforts around the world have demonstrated that fat-extracted soybean meal and soybean protein concentrates can be used to reduce or eliminate the need for fish meal in diets for many carnivorous fish and some crustaceans (Hardy, 1996, 1999). However, soybeans contain anti-nutritional factors such as trypsin inhibitors, saponins, lectins and phytate, which are not entirely eliminated by appropriate heat treatment and in salmonids have been implicated as the cause of an inflammatory, possibly antigenic, nutritional enteritis (Baeverfjord and Krogdahl, 1996; Bakke-McKellep et al., 2000). Moreover, Australia’s production of soybean and soybean meal is very small and could not sustain a major aquafeed’s industry. The other plant protein meals that are available in Australia at significant tonnages and which might be considered for prawn and barramundi feeds are cottonseed meal, canola and lupin. Of these however, canola meal was found to be poorly acceptable to prawns (severe feed rejection after 20% inclusion) and totally unacceptable to barramundi; cottonseed meal was intensely disliked by both prawns and barramundi. The reasons for this low acceptance of these meals was not investigated but most likely were caused by inherent anti-nutritional factors that they contain, particularly gossypol in cottonseed and glucosinolate in canola. The high fibre and low protein content of lupin grain reduces its suitability as a protein substitute of fish meal in Atlantic salmon, prawn and barramundi aquafeeds but dehulling to produce a lower fibre and higher protein meal has much wider application. The potential of dehulled lupin meal (Gungurru cultivar) to replace fish meal in diets for each of the target aquaculture species was tested and has showed good results. For Atlantic salmon, replacement of 25% of the fish meal protein by the lupin meal was equally as good as the fish meal-only control diet but the lupin protein was less well utilized when the substitution rate was increased to 33% (Carter and Hauler, 2000). For prawns, dehulled lupin was able to replace 20% of the marine protein in the control feed without affecting animal performance but higher substitution rates resulted in a marked down-turn in prawn growth (Sarac et al., 1998; Smith et al., 2001). For barramundi, summit dilution procedures showed that fish growth and feed conversion efficiency declined sharply with dehulled lupin meal inclusion rates greater than 30% (equivalent to a 38% replacement of animal protein) (Williams, 1998; Williams et al., 2000). While these results with dehulled lupin have been very encouraging, even more promising results are emerging with newer varieties of lupins shown to be more readily accepted by fish and prawns. At the time of doing the FMR/ADD work, supplies of plant protein concentrates were either not commercially available or were considered to be far too expensive for practical use. However, in more recent years protein concentrates of lupin and canola have become more widely available. The effect lupin variety and cultivar has on the nutritional value and acceptability of lupin meal for fish and prawns and the extent to which protein concentrates can be used as fish meal substitutes in aquafeeds will be discussed in other papers presented at this symposium.

Conclusions

Australia is fortunate in producing large tonnages of agricultural protein meals, both of plant and animal origin, that have been shown to be cost-effective partial or complete substitutes of fish meal in aquafeeds across a broad range of aquaculture species. Herbivorous species such as silver perch can use the widest range of these feedstuffs. These species are physiologically better able to digest and metabolize high carbohydrate foods and require diets with comparatively low protein and lipid content, which facilitates the incorporation of the widest selection of feed ingredients. The choice of which feed ingredient/s to use to substitute for fish meal in their diets becomes primarily an exercise of supplying digestible nutrients (and energy) at least cost. For more carnivorous aquaculture species such as tiger prawns, barramundi and Atlantic salmon, the choice of suitable alternative ingredients decreases. These species generally have a higher dietary nutrient specifications, which means that only the nutrient-rich ingredients can easily be accommodated in the diet formulation, and they are less well able to digest and/or metabolize dietary carbohydrate. Moreover, the presence of anti-nutritional factors in the feed ingredient appears to have a more profound effect on feed acceptance with carnivores than herbivores. Abattoir byproduct meals are well accepted by both herbivorous and carnivorous aquaculture species but bans on using these meals in aquafeeds in some countries effectively eliminates their use in diets for Atlantic salmon and other species that might be exported to Japan or Europe. Of the high protein grain crops that are produced in abundance in Australia, lupins and canola meals, especially those concentrates rich in protein and low in carbohydrate, have the greatest potential as substitutes for fish meal in aquafeeds.

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The Aquaculture Feed Grains Program

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Introduction

Since late 2002 the Grains R&D Corporation (GRDC) 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 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 (fish nutrition, grain processing, grain breeding, analytical chemistry, feeds processing, grain marketing etc…) 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
- Department of Fisheries
- Department of Agriculture
- Chemistry Centre

The nature of the commercial partner's involvement was to assist with progression of the commercialisation of the research and help test the realities of the research findings under specific market sector conditions. Notably, commercial partners were engaged from the full extent of the value-chain. This included grain handlers, processors and grain users. With the significant expansion of the initial GRDC funded project, and an additional FRDC funded project now included, it was agreed that under the new expanded format, with multiple component projects, that the project should be recognised as the Aquaculture Feed Grains Program (AFGP).

The program's two primary challenges were to facilitate the adoption of grains into aquaculture feeds to reduce reliance on fishmeals and to develop a new higher-value market for lupins, to try and obtain a market premium for the grain. The fishmeal replacement work built on from the highly successful FRDC Fishmeal Replacement Subprogram of the 1990's and engaged several of the partners from that subprogram as well as utilising key findings to target and fast-track its efforts. The work from the FRDC Fishmeal Replacement Subprogram identified lupins, soybean, peas and rapeseed meals as all having some potential as feed grains for the aquaculture sector.

Grain Production and Market Trends

Lupins were one of the key grain varieties identified in the work of the FRDC Fishmeal Replacement Subprogram. However, uptake of use of grains by the aquaculture feed sector had been slow despite this progress. Because of Australia dominance in lupin production is was realised that any gains made in development of this grain variety would be predominantly captured within Australia, as opposed to soybean, where the benefit would primarily flow to North and South America. In addition to this there was a need to improve profitability of lupin production. With relatively low grain prices and relatively low yields, lupins were an expensive crop to produce. To address this substantial effort has been spent on improving crop yields, as well as improving grain characteristics.

Annual lupin production over the past five years (2000 – 2005) has averaged at 996,500 tonnes per annum (Wilkins and Robertson, 2005) (Figure 1). As of 2005, Chile is the next largest producer of lupins, although the EU combined with former Soviet states is also a significant producer with an annual crop of around 83,000 tonnes per annum. In Australia 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 (Figure 1). 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

Domestic lupin use in WA is limited and there is limited lupin trade between Eastern and Western Australia because of quarantine and logistical issues. 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 (Wilkins and Robertson, 2005).

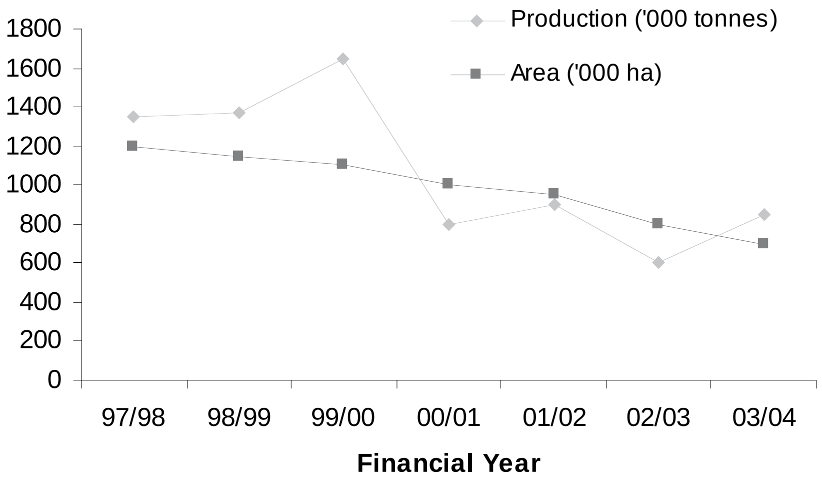

Figure 1. Variation in lupin production in Western Australia from 1997 - 2004.

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 (Table 2). In contrast to the Eastern states of Australia, the lack of a significant domestic market in Western Australia means that grain marketing is heavily export directed.

Global production of lupins, at around 1.5 million tonnes, is tiny compared to annual soybean meal production of around 125 million tonnes. Based on this production volume, the prospect for the lupin kernel meal market decreases 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 “point-of-difference” features between lupin kernel meals and soybean meal use in the salmonid aquaculture feed sector have now been identified and it is hypothesised that there may be other “point-of-difference” aspects that are yet to be identified. Notably, it was only through targeted R&D focussing on particular grain and particular aquaculture sectors that these “point-of-difference” aspects were identified.

Table 1. Volume of production of key grains in Australia by variety and total product by state (avg. 2000 – 2005).

Grain	Tonnes (Million)	State	Tonnes (Million)
Wheat	20,514	WA	11.517
Barley	5,862	NSW	10.737
Sorghum	1,806	SA	6.255
Chick-Peas	195	VIC	4.668
Lupins	1,294	QLD	2.905
Field Peas	371	National	36.082
Canola	1,687		
Triticale	688		
Oats	1,225		
Faba Beans	241		

Data from ABARE Crop reports 2005.

Which Aquaculture Markets and Why ?

Initial efforts to develop new markets for lupins focused on Southeast Asian species and had limited adoption. In 2000 a review was undertaken of the strategy to target Southeast Asian aquaculture feeds and the review identified that the premium aquaculture feed sector, such as salmon feeds, would be more likely to pay a premium for the use of lupins – if "point-of-difference" aspects could be determined with respect to the market price setter of soybean meal. This aquaculture sector also had fewer protein alternatives available to it and was more performance-sensitive than cost-sensitive. This was a contrast to the tilapia and catfish industries in Southeast Asia, which are highly cost-sensitive and are not so performance-sensitive industries. It was also recognised that the Southeast Asian industries also tended to be technology followers not developers and early-adopters like the salmon industry. Therefore by focusing development attention on the international salmon feed sector the other fish industries would be likely to take on the technology once it had gained acceptance in that aquaculture sector.

In addition to this, salmonid (Atlantic salmon and rainbow trout) production was the largest aquaculture industry in Australia using formulated feed technology (Table 2). The other two large formulated feed sectors being prawns and barramundi. Tuna, while being the largest feed user of the aquaculture industries remains reluctant to adopt pelleted feed technology and therefore was not considered a worthwhile sector to consider in the research program.

Table 2. Feed use estimates of key Australian aquaculture industries in 2001 and 2005.

	2000	2005
Salmon	19,086	21,608
Trout	2,340	2,296
Tuna	135,765	111,885
Perch	428	471
Barramundi	1,347	2,645
Prawns	5,074	5,821
Yabbies	552	240
Marron	120	154
Total Extruded	23,201	27,019
Total Pelleted	5,746	6,215

Values based on production tonnages (ABARE – Australian Fisheries Statistics 2005) x Food conversion ratios x Correction factor to account for production time (i.e. 18 – 20 months to grow salmon to harvest).

To address the primary challenges of the program two-key aquaculture species of Atlantic salmon and prawns were chosen to be used in evaluating the value-added grain products produced from the other parts of the program. In other parts of the program product screening and development work was conducted with rainbow trout as a "lab-rat" species and cross-referencing it with other species has shown that it is highly a suitable model, particularly for Atlantic salmon. In addition to the earlier identified reasons, these two markets were also 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.

Although lupin kernel meals and protein concentrates have been shown to be able to be included in diets up to 40% (Farhangi and Carter, 2001; Glencross et al., 2004) and in doing so replace up to 75% of the fish meal content of the diet without palatability or growth problems, there is little practical application for such high inclusion levels. Typically more realistic commercial inclusion levels for practical feeds are of the order of 10% to 20% depending on price and protein content. While higher inclusion levels would be feasible in diets for tilapia and catfish species this was not earmarked as a target market for prior mentioned reasons. 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 as is the interchange-ability of raw materials in these diets (Table 3).

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	250	300	450	450
Diet Fat	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.

Program Targeted Objectives

To address the program's challenges a series of specific objectives were developed. These included:

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
3. Commercial transfer of intellectual property for development of new grain product(s)

To service these objectives a series of components were initiated that covered a broad area of grain and aquaculture feed issues to underpin the development for a potential lupin market within the aquaculture feed sector. These components and their intentions were:

- Grain Processing – Identify and develop new grain varieties and products with feed application
- Grain Varieties – Provide input to grain breeding programs on end-user requirements
- Biological Assessment – Evaluate new and emerging products to gain technology on end-user requirements
- Feed Extrusion – Develop extrusion capacity and grain product technology
- Technology Extension – Develop broad grain and feed industry networks and promote lupin products

Specific outcomes relating to each of these components will be detailed in other parts of the workshop/ proceedings.

Industry Progress

Since the initiation of the program, several major industry initiatives have taken place. Notably, Skretting Australia was one of the pioneers in the utilisation of lupin products in its feeds and this advance has spread throughout its parent company across the world. As a consequence of the adoption of lupin kernel meal by Skretting, other competitor companies such as Ridleys, BioMar and EWOS have also taken strong interest in this work, particularly in Norway and Chile. Promotional visits have been undertaken in 2004, 2005 and 2006 to visit these companies in Australia, Norway, Scotland and Chile to promote lupins and provide the foundation for future visits by Australian grain marketers.

One of the key issues identified by Skretting Australia, as a partner in the program was the issue of variability in the composition and its potential implications on the nutritional value of the raw material. To address this issue a major part of the program’s activities were directed towards the development of quality assurance (QA) technology in the form of Near Infrared Spectroscopy (NIRS) technology for rapid analysis of crude composition and digestible protein and energy values. This comprised the collection of 75 samples of lupin kernel meal and their respective digestibility evaluation through rainbow trout. Each of the 75 samples were then provided to the three commercial partners (Weston Technologies, CBH-Group, Skretting Australia) along with the composition and digestibility data for each to develop their own calibrations for their own NIRS equipment. Development of NIRS calibrations within the program, at the Department of Agriculture and Food has resulted in a viable, but preliminary calibration for the composition analysis of lupin seed and kernel meals. Development of the calibration for digestible value of the kernel meals has so far proven non-viable and requires further samples to improve its viability.

During the term of the program, George Weston Foods and CBH-Group also announced the development of a joint-venture project, Australasian Lupin Processing Pty Ltd. This facility, based in Perth at the Metropolitan Grains Centre (MGC) was built in 2005-2006 and is to be commissioned early in 2007. It has the capacity to process 200,000 tonnes of lupins to kernel meal. It was constructed with the specific intention of one its key target markets for its products being the national and international aquaculture feeds industries. Further value-adding of lupin kernel meals to make lupin protein and fibre isolates and concentrates has also been touted. This technology is also another component that the joint-venture has capitalised on through being a partner in the AFGP.

In 2003 a significant trial batch of yellow lupins was used by the aquaculture feed industry with highly promising results. Small consignments of this variety continue to be used in the industry, but the major bottleneck is in the production of significant volumes of grain to supply this sector. While new varieties of yellow lupins (Pootalong™) have been released in recent years, a major breakthrough is still required in terms of yield or significant volumes of grain to create a viable industry sector. In 2006 a new lupin variety Coromup™ was released. This new variety is one of the first higher-protein varieties to be released and reverses a trend of decreasing protein in lupins that has been occurring over the past 10 years.

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.

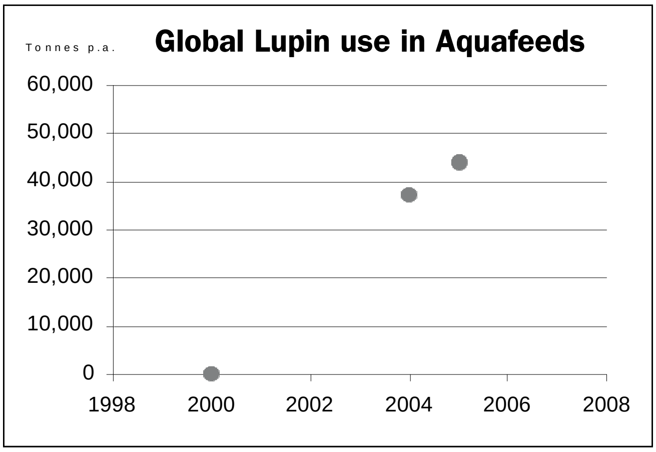

Figure 2. Volume of lupin kernel meal use in aqua-feeds internationally since 1998.

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 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*). Anecdotal data obtained on visits to Chile suggests that the volume of lupins used in Chile may be higher than 20,000 tonnes. 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. While most lupin use has been for salmon feeds, some lupin use has also occurred throughout the world in feeds for trout, barramundi, yellowtail kingfish and red seabream.

In 2007 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 primarily by the drought of 2006. The drought has resulted in limited stocks of lupins being available and what lupins are available are obtaining very high prices in the feedlot and dairy industries due to a lack of hay fodder.

From the experience of seeing lupin kernel meals penetrate into the aquaculture feed market over the past few years it has been possible to assess the potential impact that each feed market will have on price paid per tonne of lupin seed produced by the farmer (Table 4). Based on a kernel yield of 70% and a price of $350 per tonne (f.o.b. basis) a seed value of $245 per tonne of seed is realised for lupins sold into the aquaculture feed sector. If the by-product of the hulls is on-sold to feedlot or dairy industries, then a total seed value of $275 per tonne of seed is achieved. Both of these values are significantly higher than the next best option of seeds to feedlot/dairy (in non-drought years) where $220 per tonne of seed is achieved. Notably, both pigs and poultry tend to be options of last resort as the lysine to digestible energy ratio is poorer than many other raw materials, making them less desirable than other raw materials and also for poultry there are inclusion limitations associated with sticky droppings.

Table 4. Relative prices paid in WA for lupins according to market sector and grain fraction.

Market	Seed value	Kernel value	Hull value
Human food	?	?	?
Aqua + Feedlot	275	350	100
Aqua	245	350	-
Feedlot	220	-	-
Purchase Price	200	-	-
Pigs	190	-	-
Poultry	180	-	-

Approximate values obtained from industry sources. Values assume non-drought conditions in Australia.

Although the price obtained for promoting lupins into the aquaculture feed sector will deliver a price premium, whether this premium gets returned to the grower will be dependent on several things. One option is for the grower to establish their own value-chain and thereby capture price premiums directly. Alternatively there would have to be a significant increase in the proportion of the total lupin volume of the lupin crop going into aquaculture feeds to move the average price enough to warrant a change in the pool price. This however will be highly dependent on international soybean meal prices and exchange rates and makes it very difficult for significant price gains to be achieved through a pooling option.

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Feed Grain Constraints – An Industry Perspective

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Introduction

Skretting is the world leader in production and supply of feed for salmon and trout, meeting 40% of global demand. Total annual production of Skretting feed is more than one million tonnes and provides nutrition suited to over 50 species of farmed fish worldwide. For future sustainability of global aquaculture, companies like Skretting need to decrease dependence on fish meal and fish oil, and make investments that achieve increased grain use in fish feed.

In the European Union (EU), legislation restricting land animal material in feeds puts an emphasis on the use of grain ingredients as fishmeal replacements. In other parts of the world, grain usage is required to supply the starch for manufacturing purposes, but these diets will typically contain less diet grain ingredients overall.

Energy density of feed also dictates the required nutrient density of the grain ingredient itself. Salmonid diets have the highest energy densities (typically 39% protein and 36% oil, > 22 MJ DE per kg) and require nutrient dense ingredients. Marine species diets are typically less energy dense, and can make use of less dense nutrient dense ingredient.

Soybean meal and corn gluten meal are the most widely traded grains (or grain products) globally, and have reasonably high nutrient density. Consequently these ingredients are commonly included in salmonids diets, particularly in regions where land animal protein use is restricted. In addition to this, protein concentrates from soybean meal, wheat and to a lesser extent, canola (rapeseed) are also used. Dehulled lupin meal, dehulled sunflower meal and extracted canola (rapeseed) meal are considered in salmonid recipes when competitive. Lower energy dense marine species diets make use of all these ingredients, along with less nutrient dense ingredient such as dehulled peas and beans.

On the whole, these grain materials are digested well by all fish species - and ultimately it is the digestible nutrient density that dictates suitability in modern fish feed formulation.

A further restriction on the use of grain in fish feed is the presence of anti-nutritional factors. Considerable resources are dedicated to developing solutions for the most specific grain materials, with considerable success in recent years.

Very high fishmeal replacement with grains will cause palatability issues in both salmonids and marine species. Palatability enhancers can be applied to compensate, but current commercial products have only short-term feed intake benefits.

To maintain sustainability of global aquaculture there will be a continued focus on fishmeal replacement using whole grain ingredients and grain ingredient refined specifically for the aquaculture sector.

Strategies for Assessing Feed Grains for Fish

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Introduction

Modern aquaculture diets are complex assemblages of a range of raw materials (formulation) processed to make a feed that satisfies the demands of the animal for nutrients and energy (specifications). In most modern fish diets, the specifications are now often prescribed on a digestible nutrient and energy basis. In the formulation process not only are the diet specifications a critical aspect to the diet, but also the cost of the formulation, its process-ability (ability to make it into a pellet) and also the consideration of the potential for certain raw materials to introduce deleterious biologically active compounds, are all important issues.

The assessment of different feed grain options for use in fish diets can be undertaken using several different strategies. While there is seldom one perfect strategy to provide all answers, there are certain methods that can provide more useful data than others for the formulation of cost-effective, viable fish diets (Glencross et al., 2007). Generally knowledge on raw materials in feeds for fish is gained by producing feeds with the raw material in question being varied, and the feed then being fed to fish in a variety of treatments. The strategy used for diet design is also open to several strategies. A range of assessments to this feeding process can then be applied. Generally there are five loci where the effect of a feed on fish can be routinely assessed, and by inference the effect of a raw material, depending on the feed formulation and specifications (Figure 1). These five loci can be considered as: (1) feed intake, (2) utilisation, (3) pathology, (4) blood chemistry, and (5) digestibility. Of these five the assessment of digestibility, palatability and utilisation are the primary loci of importance in the assessment of raw material effects. If there are subsequent aberrations determined in these loci, then further assessment of the others is sometimes used to determine specifically what is going on.

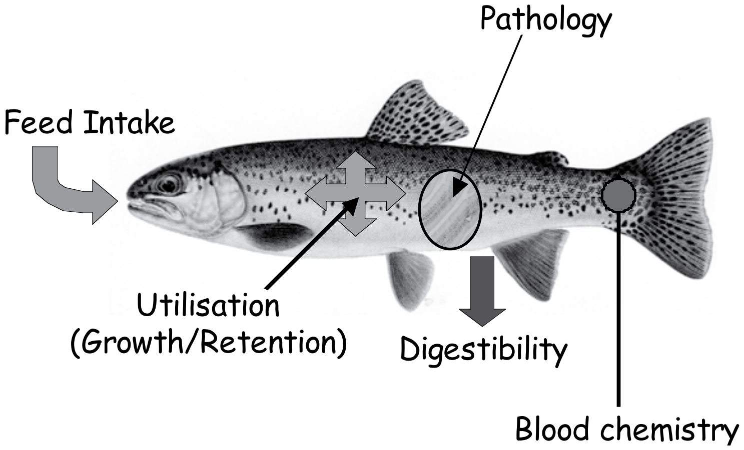

Figure 1. Key loci for studying the interaction of a feed with fish.

Methods of Assessment

Digestibility of diets, and by inference certain component raw materials, are one of the more practical assessment methods used in aquaculture nutrition research. There are a range of methods that can be used to determine the digestibility of raw materials and key considerations include the diet design and faecal collection method used (Glencross et al., 2007). There has been considerable debate over the more relevant method to use in the collection of faeces for the assessment of digestibility in fish (Vandenberg and de la Noue, 2001). However, the use of faecal stripping techniques is accepted as a more conservative technique in digestibility assessment and because of this has gained more widespread acceptance as a faecal collection technique for commercially used data (Glencross et al., 2005).

Provided a diet is formulated on a digestible nutrient and energy basis, and it satisfies the animal's nutrient and energy requirements, most variability in performance is then usually attributable to vagaries in feed intake. Therefore, armed with knowledge on digestibility and palatability of a raw material, its potential value becomes substantially clearer. To determine the palatability of a raw material the effect that it has on feed intake needs to be assessed. Again this is dependent on diet design, but generally involves the addition of the raw material in question, in increasing levels, to a reference diet (Glencross et al. 2006). The diets are then fed to tanks of fish and the feed intake determined based on the amount fed minus the collected uneaten feed (Helland et al., 1996). The feed intake variability is well known to be most sensitive within the first week of introduction of a feed to fish and therefore this period, prior to adaptation, can be used to better discriminate palatability effects (Wybourne and Carter, 1999). The final assessment of feed intake effects can be examined either on a pair-wise time-series basis or simply as a comparison of differences in absolute intake over a period of time (Glencross et al., 2006).

Utilisation can be measured in several ways (Glencross et al., 2007). One of the most obvious ways is to assess the weight gain achieved when fish are fed certain diets. In this regard, fish are fed a particular diet for a certain period of time and their weight gain measured between two time points after being fed on a diet. Comparisons can then be made among treatments and inferences made according to what the diets where. Further detail can be added to this style of assessment by examining the changes in composition of the animal, for example protein and/or energy (or indeed any nutrient) as a function of the intake of protein or energy (Cho and Kaushik, 1990; Lupatsch et al., 2003). This is referred to as the protein or energy retention efficiency. Assessment of fatty acid demands and utilisation of alternative oils resources in particular requires considerable focus on the utilisation aspects of the diets (Glencross et al., 2003). There are many variants that can be applied on the general principles of utilisation studies and all have some value depending on the questions being asked. The different strategies have been reviewed in Glencross et al. (2007).

In some instances the assessment of digestibility, palatability and utilisation will identify problems. In some instances further assessment strategies are required to determine why such problems are occurring. Pathology assessment using histology is one option. By examining the changes in cellular structure of certain organs from fish from certain treatment, relative to those from control treatments the effects of raw materials on certain pathologies can be determined. For instance the assessment of soybean meal inclusion on the cellular structure of the distal intestine of Atlantic salmon has been shown to cause a condition referred to as 'distal enteritis' (Refstie et al. 2006). A histological assessment can also be used to provide some assurance that no organ damage is occurring with the inclusion of certain raw materials, and a focus on gut, liver and kidney tissues can provide some assurance of whether-or-not any damage at a cellular level is occurring (Glencross et al., 2006a).

The assessment of blood chemistry can provide a further avenue for understanding the effects of raw materials. Parameters such as hormones, glucose, amino acids among others can all be evaluated. In some cases the observations need to be regarded according to species and feed intake to ensure the correct interpretation is arrived at.

Irrespective of the assessment method used, the appropriate diet design is central to the effective assessment of raw material implications in fish diets. However, the strategic assessment of digestibility, palatability and utilisation features of any raw material can provide a sound basis for determining its potential in an aquaculture feed.

Extrusion Processing

Because raw materials have to be combined to satisfy both nutritional and physical constraints of feeds it is important that the effect that raw materials have on feed processing are also considered (Kaushik, 2001). Most modern aquaculture feeds for fish are produced using an extrusion process (Hardy and Barrows, 2002). Because of this it is pertinent that the raw materials being studied are also assessed using similar technology.

A range a strategies can be used to assess the effect of raw materials on extrusion, but the simplest involves the addition of the raw material being considered to a reference diet and it being processed using fixed operating conditions. Other alternatives include adding the raw material, but maintaining a fixed composition, or even varying the operating conditions to determine the optimal processing parameters required to work with certain raw materials (Evans, 1999).

Whatever the strategy used to process the diet, the pellets produced still require some form of assessment of their physical properties to allow the effect of the raw material to be determined.

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Lupins in Aquafeeds

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Introduction

Lupins are the harvested seed of species from the *Lupinus* genus, a group within the leguminous bean and pea family Fabaceae. Legumes are particularly valuable agricultural crops because of their capacity to provide a grain crop and also fix and return atmospheric nitrogen to soils and improve the soil value for further cropping. The oilseeds, soybeans and peanuts are also leguminous plants, though traditionally they have been cropped for their oil value, whereas lupins are cropped for both their protein and nitrogen fixing value. There are three primary commercial species of lupins, *L. angustifolius* (Narrow-leafed Sweet Lupin), *L. albus* (White or Albus Lupin) and *L. luteus* (Yellow Lupin). *L. angustifolius* dominates world lupin production.

Lupins are the largest of the legume crops grown in Australia. Production in Australia has a five-year average of around 1,300,000 tonnes per annum, which constitutes about 88% of global lupin production. Typically lupins are grown in rotation with other crops such as wheat, barley and canola in the Australian grain belt. The majority of Australian grown lupins are produced in the south-western region of Australia. This region produces about 85% of all Australian lupin production.

Production is dominated by *L. angustifolius*, which constitutes over 95% of all tonnage. Both *L. albus* and *L. luteus* make up the remainder of the lupin species grown. Some experimental work is proceeding with the South American species of *Lupinus mutabilis,* including preliminary feeding trials with fish.

Production of *L. angustifolius* dominates because of its higher yielding and disease tolerance characteristics compared to the other lupin species. Concerted effort on the development of improved genetic lines using conventional breeding approaches has been undertaken on since the 1980's. Initially, key breeding criteria were reduction in alkaloids, disease resistance and yield. Protein level has been introduced as another selection trait for breeding programs. A new, higher-protein variety was released in 2006.

Compositional Features of Lupin Meals

There is considerable variability in the composition of lupin meals depending on both lupins species and whether they are in a whole-seed or kernel form (Table 1). Protein content of lupins is significantly increased by the production of a kernel (seed coat removed/dehulled) form. For example *L. angustifolius* increases from 32% to 41% protein through dehulling. Protein content of lupins is highest in the kernel meals of *L. luteus* and *L. mutabilis* varieties, which both exceed 50% protein in the kernel meal form. Fat content is highest in the *L. mutabilis* and *L. albus* varieties. The composition of *L. mutabilis* is similar to that of soybeans. Lupins are notable in that they possess negligible starch levels within their carbohydrate component. Lignin is also present in very low quantities.

Table 1. Gross chemical composition (%) of the four lupin species *L. albus, L. luteus, L. angustifolius, L. mutabilis.* Derived from Sipsas (2003).

Species	***L. angustifolius***		***L. albus***		***L. luteus***		***L. mutabilis***	
	Seed	**Kernel**	**Seed**	**Kernel**	**Seed**	**Kernel**	**Seed**	**Kernel**
Seed Coat	24.0	0.0	18.0	0.0	27.0	0.0	16.0	0.0
Moisture	9.0	12.0	9.0	11.0	9.0	12.0	9.0	12.0
Protein	32.0	41.0	36.0	44.0	38.0	52.0	44.0	52.0
Fat	6.0	7.0	9.0	11.0	5.0	7.0	14.0	17.0
Ash	3.0	3.0	3.0	4.0	3.0	4.0	3.0	4.0
Lignin	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Polysaccharides	22.0	28.0	17.0	21.0	8.0	10.0	9.0	10.0
Oligosaccharides	4.0	6.0	7.0	8.0	9.0	12.0	?	?
Minor Components	0.5	1.0	0.6	1.0	0.9	1.0	?	?

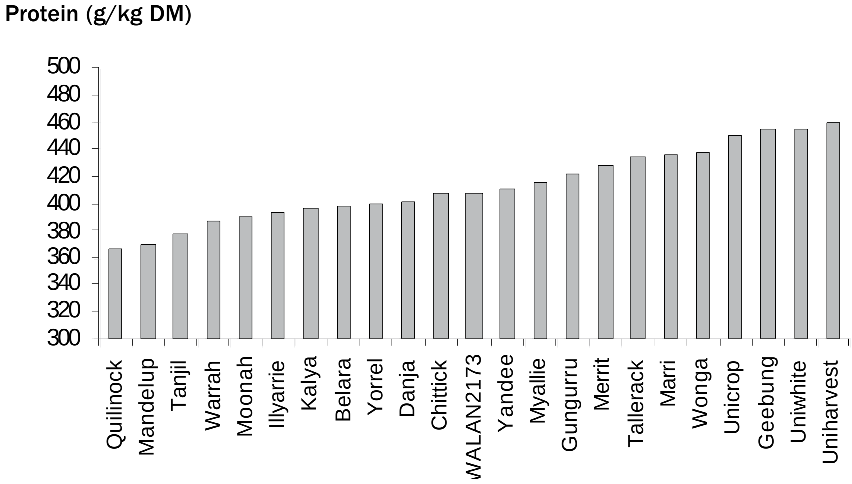

Figure 1. Crude protein content of different kernel meals of cultivars of *L. angutifolius.*

Within the different lupin varieties significant variability in protein composition has been observed (Sipsas and Glencross, 2005). There are both genetic and environmental factors that influence this composition variability (French, 2005). The drier cropping regions have been shown to more regularly produce higher protein varieties, though managing farm inputs to achieve increase protein levels has proven difficult. Genotype (cultivar) choice has been the most viable option so far and this has resulted in recent releases of higher protein *L. angustifolius* varieties such as cv. Coromup (tested as WALAN2173). This variability in protein levels, when managed appropriately has the capacity to significantly enhance the value of lupins as a feed grain for aquaculture. Provided a segregation mechanism is introduced it also has the potential to improve price returns for farmers (Kingwell, 2005). However, the lack a current segregation system has driven lupin production to be yield rather than quality driven and the majority of production is now of lower protein varieties such as Mandelup, Tanjil and Belara.

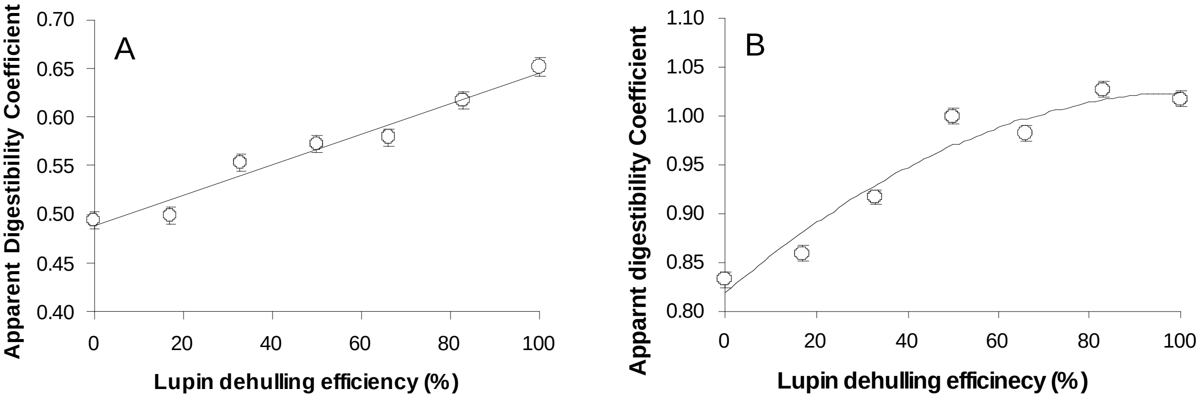

Figure 2. Digestibility of energy (A) and protein (B) from lupins with increasing dehulling efficiency. Notable is the significant improvement of both protein and energy digestibility with increasing removal of the seed hull content from the meal.

Digestibility of Lupin Meals

In a study that examined the effect of dehulling efficiency on protein and energy digestibility, the benefits of dehulling were clearly demonstrated (Figure 2). Taking a single-source batch of *L. angustifolius* seed, the seed was divided into two batches, one batch milled as whole-seed, while the other batch was dehulled to >99.9% purity with manual cleaning of the kernels following dehulling, prior to milling. The two batches were then blended against each other to create in effect a series of meals representing different dehulling efficiencies. The results showed that energy digestibility responded linearly to the dehulling efficiency, while protein digestibility responded curvilinear to dehulling efficiency. One feature that this work identified was the importance of kernel meal preparation on the digestible value likely to be achieved from lupin kernel meals.

The digestibility of the kernel meals of all three species of lupin (*Lupinus albus, L. angustifolius* and *L. luteus*) was compared against each other and a reference ingredient of solvent extracted soybean meal, when fed to rainbow trout (*Oncorhynchus mykiss*) (Glencross and Hawkins, 2004). The digestibility of protein of all lupin kernel meals was better than for the soybean meal. The highest protein digestibility was that from *L. luteus* kernel meal (100%), which at similar inclusion levels was better than that from kernel meals of both *L. albus* (96.7%) and *L. angustifolius* (95.3%) and also the soybean meal (86.7%). The digestibility of dietary energy from each of the lupin kernel meals (range from 70.1% to 74.2%) was less than that obtained from soybean meal (79.8%). However, the higher gross energy content of the lupin kernel meals still resulted in both *L. luteus* and *L. albus* providing greater levels of digestible dietary energy with their inclusion in the diet than the inclusion of soybean meal would provide. Indeed it was suggested that both of these ingredients would already constitute appropriate replacements of any soybean meal used in diets for salmonids, particularly in lieu of the cost competitiveness. These findings were reinforced by subsequent work using refined methodologies (Glencross et al., 2005).

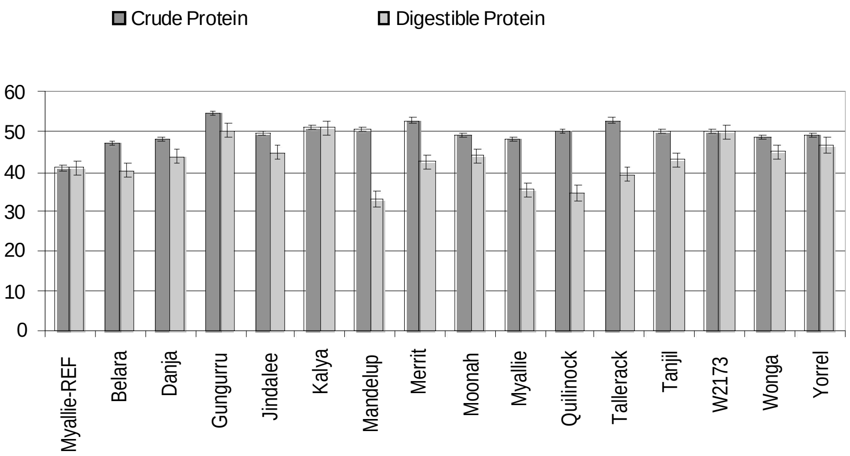

Figure 3. Digestibility of protein and energy from lupin kernel meals according to *L. angustifolius* cultivar type. Notable is the significant variability among the different cultivars and even within cultivars from different years and locations.

The variability in the digestibility of protein and energy of different cultivars of *L. angustifolius* kernel meal when fed to rainbow trout was initially studied by Glencross et al. (2003a). In this work these authors found that there was substantial variability in digestibility of both protein and energy from lupin kernel meals and that this variability correlated well with crude protein content of the meals. This work was followed up with a more intensive study that found limited correlation between protein and energy from lupin kernel meals and crude protein content of the meals (Figure 3; Glencross et al., 2006b). With the issue unresolved it was decided to increase the data set to a significant number that may enable the development of a calibration set for near infra-red spectroscopy (NIRS) and accordingly six experiments with 75 different lupin kernel meal samples were assessed for their digestibilities in rainbow trout (Figure 4).

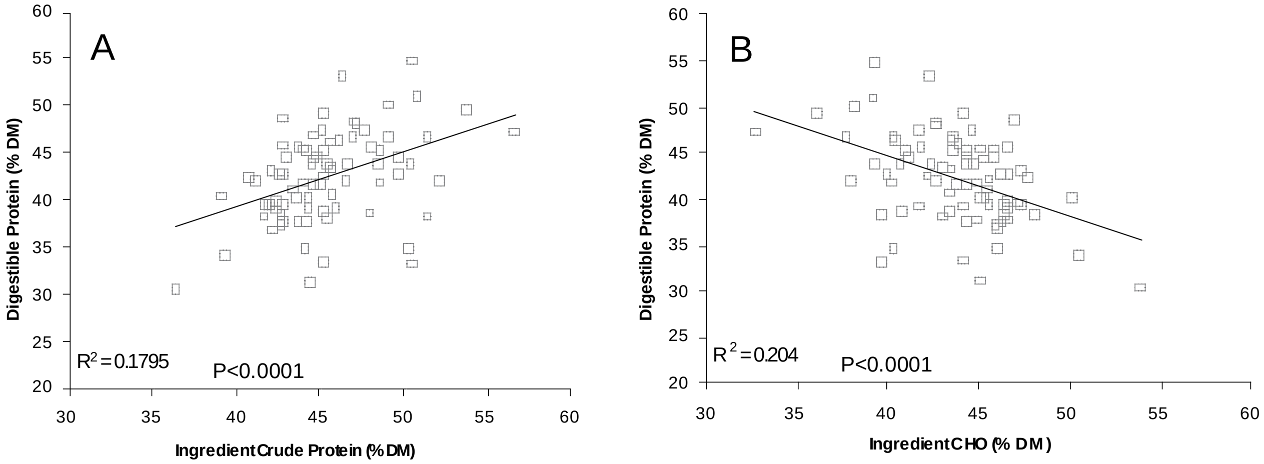

Figure 4. Digestible protein level of lupin kernel meals as influenced by crude protein level (A) and crude carbohydrate level (B). Notable is the significant relationships between kernel meal crude protein content and carbohydrate content and the digestible protein level of that kernel meal.

The larger data set confirmed the findings of the initial preliminary study, in that the crude protein level in the kernel meal does influence the protein and energy digestibility of the kernel meal, and by the inverse of this a higher carbohydrate content of lupin kernel meals has a negative influence on digestible protein and energy levels. This data-set, the largest of its kind ever undertaken for a feed grain for aquaculture, is still being evaluated for further features that may refine our understanding of the factors that drive nutritional value of feed grains in fish. The influence of different fibre classes is one additional factor presently being explored. An important point that this follow-up work demonstrated was the risk associated with being reliant on small data-sets to formulate hypotheses of this nature. Presently the development of a NIRS calibration for digestible protein and energy is still in progress, but it appears that additional samples will be required to produce a reliable calibration. Calibration development of digestible energy value is appearing the most viable from the data so far.

This aspect of nutritional assessment of lupins was also observed in work done on the development and assessment of protein concentrates developed from lupins (Figure 5). In an assessment of the digestibility of protein concentrates and isolates produced from lupins it was shown that with increasing protein concentration that there were substantial improvements in energy digestibility, but little impact on the protein digestibility. This effect was also similar for soybean products (Glencross et al., 2004b).

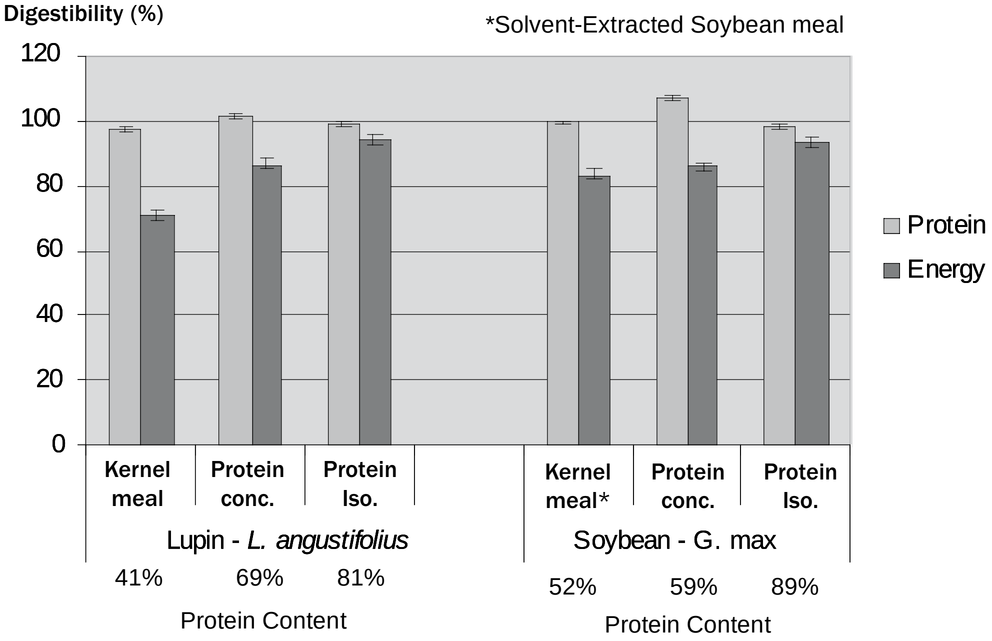

Figure 5. Digestibility of protein and energy from lupins and soybeans with increasing level of protein concentration. Notable is the significant improvement of energy digestibility with increasing protein content of the protein source, but limited improvements in the protein digestibility. From Glencross et al. (2004b).

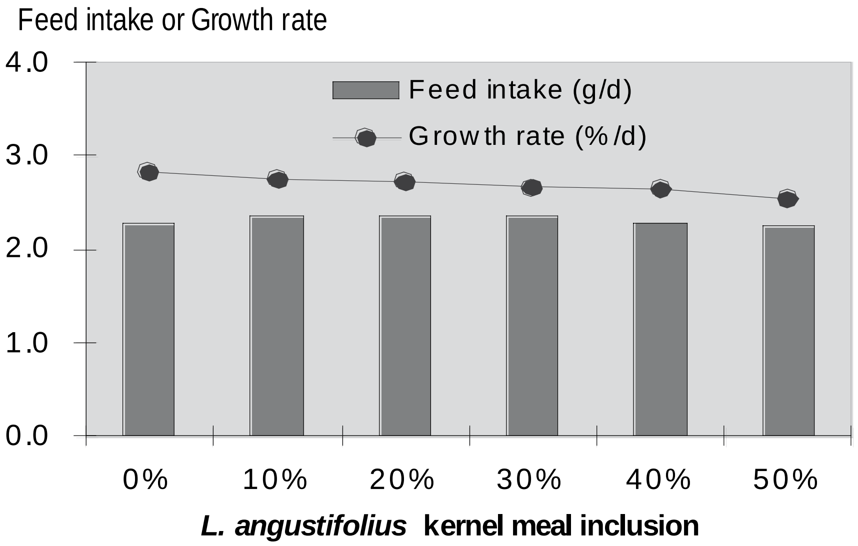

Figure 6. Growth and feed intake response of rainbow trout to increasing dietary inclusion of *L. angustifolius* kernel meal (Farhangi and Carter, 2001). Notable is the slight reduction in growth as inclusion level increases, but no decline in feed intake even at the highest inclusion levels.

Growth Studies with Lupins

The serial inclusion of *L. angustifolius* kernel meal in diets fed to rainbow trout was studied using 10% increments up to an inclusion level of 50% (Figure 6; Farhangi and Carter 2001). Using regression analysis the authors concluded that growth deteriorated at each inclusion level of the *L. angustifolius* kernel meal, though significant differences between treatments and the 0% reference were only observed at the highest inclusion levels (40% and 50%). Feed intake throughout the study was consistent and showed no effects of lupin inclusion, supporting that the diets were palatable up to and including the 50% lupin diet.

A similar experiment examined the serial inclusion of yellow lupin (*L. luteus* cv. Wodjil) kernel meal in diets fed to rainbow trout (Figure 7; Glencross et al., 2004b). The diets were formulated on an equivalent digestible protein and energy basis and fed to apparent satiety. It was found that growth was significantly reduced at 50% inclusion relative to the 0% reference diet, but not at any other inclusion level. Similar to the findings of Farhangi and Carter (2001), regression analysis suggested that there was a decline at each inclusion level though and this slight deterioration in performance was only exhibited as a significant effect at the highest inclusion level. Given that the original digestible values were based on an inclusion level of 30% then higher levels may in fact have had reduced protein and energy digestibilities. This effect would explain the reduced growth and also the observed linear relationship between the serial inclusion levels of growth performance.. Notably, feed intake was not influenced by *L. luteus* kernel meal inclusion level.

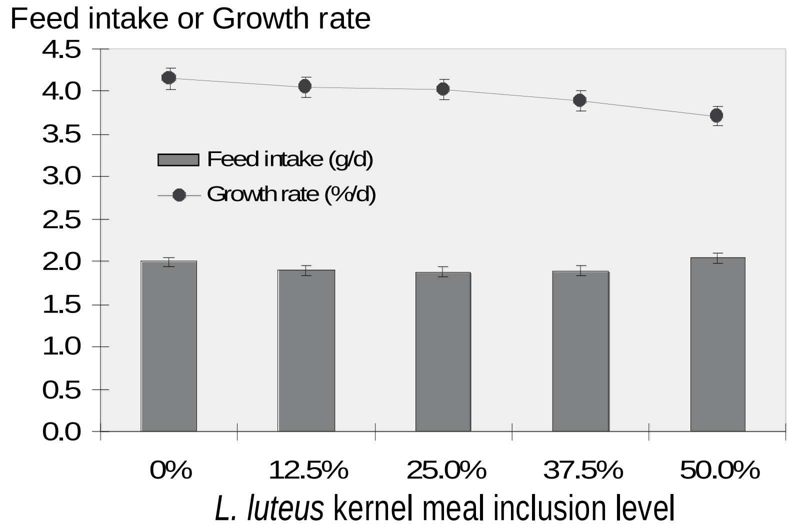

Figure 7. Growth and feed intake response of rainbow trout to increasing dietary inclusion of *L. luteus* kernel meal (Glencross et al., 2004b). Notable is the slight reduction in growth as inclusion level increases, but no decline in feed intake even at the highest inclusion levels.

Using a bio-energetic approach the utilisation efficiencies of fish fed diets with increasing levels of *L. angustifolius* kernel meals inclusion was studied to determine whether the inclusion of lupin protein significantly reduced the efficiency of protein utilisation by fish (Figure 8). Diets were formulated and prepared on an equal digestible nutrient and energy basis and fed to rainbow trout at one of four ration levels. The gain of protein or energy was then determined against the intake of digestible protein or energy. In this study it was found that the inclusion of lupin kernel meal did not affect the utilisation of dietary digestible energy or dietary digestible protein. This observation supports the hypothesis that this grain protein source is as effective an energy and protein source as fishmeal when considered on an equivalent digestible basis. This in effect proves that fish can utilise plant proteins as effectively as they can use fish based proteins.

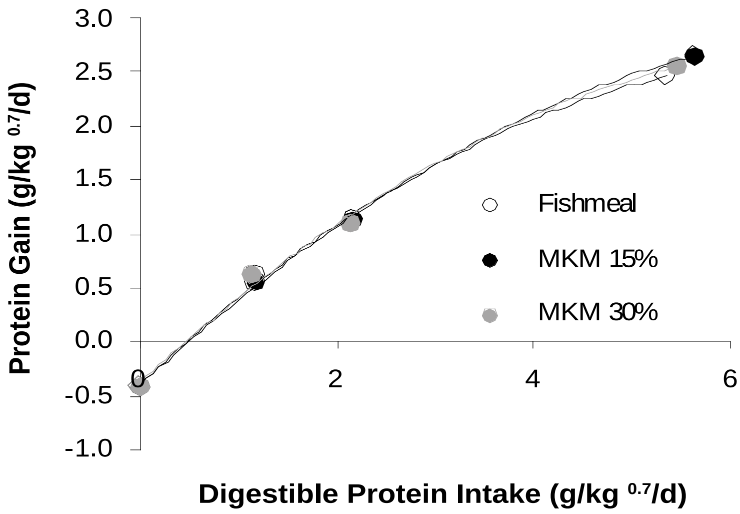

Figure 8. Protein utilization by rainbow trout of diets containing either of two inclusion levels of *L. angustifolius* (MKM) kernel meal. Notable is that the addition of lupin kernel meal does not change the efficiency at which fish utilise their dietary protein. This in effect provides proof that fish can utilise plant proteins as well as they use fish meal proteins.

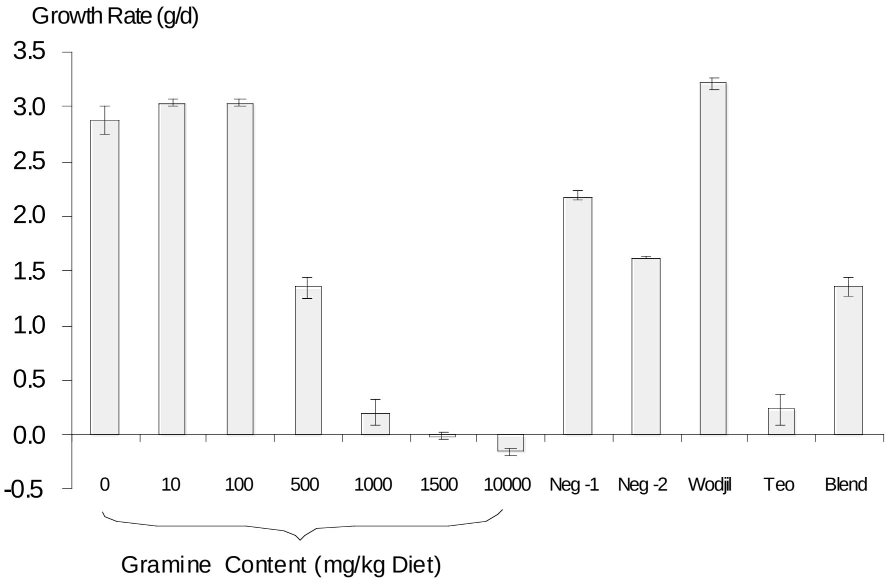

Figure 9. Response of rainbow trout to dietary levels of the lupin alkaloid gramine. Notable is the dramatic decline in growth between 100 mg/kg and 500 mg/kg associated with a decline in feed intake. Similar corresponding effects were noted from L. luteus kernel meals with varying alkaloid levels.

Anti-Nutritional Factors and Lupins

Lupins, like all members of the Legume plant family, contain certain anti-nutritional factors (ANF). These compounds are biologically active substances produced by plants, essentially as chemical defence mechanisms. A wide variety of ANF compounds exists, with equal variety in their occurrence and concentration among the different feed grain varieties. Key anti-nutritional present in lupins include alkaloids and oligosaccharides. Notably phytate, saponins, tannins, protease inhibitors and lectins, are comparatively lower than other grain legume varieties. The mode of action of these ANF also varies, with some affecting palatability, others digestion and some interfering with metabolic rate (Francis et al., 2001).

The influence of the alkaloid gramine was examined when included in diets fed to rainbow trout (Glencross et al., 2006a). It was observed that the critical level for feed intake reduction and reduced growth was between 100 – 500 mg/kg diet (Figure 9). Fish did not adapt to gramine inclusion in their diet, with feed intake reduction being maintained for the duration of the experiment. This work also showed that diets containing the current Australian commercial *L. luteus* variety (cv. Wodjil) had no alkaloid related problems, but that diets incorporating an older, parental variety (cv. Teo), had poor feed intake and subsequently poor growth. Histological observations were consistent with the degree of starvation noted at each relative gramine inclusion level, with no other metabolic aberrations or specific histological damage noted.

The influence of lupin oligosaccharides on protein, energy and organic matter digestibility has been examined in diets fed to rainbow trout (Glencross et al., 2003b). Using a cross-referencing method of both chemical and enzymatic removal of oligosaccharides, it was demonstrated that the oligosaccharide content of lupins did exert a negative effect on digestibility of protein, energy and organic matter. However, the effects were not dramatic though and closely controlled conditions, with high lupin inclusion levels were required to obtain an experimental effect. Practically, it was demonstrated that it was unlikely that *L. angustifolius* oligosaccharides were likely to cause significant ANF problems to fish.

Recent work with Atlantic salmon (Refstie et al., 2006) has also demonstrated that there is a significant lack in both *L. angustifolius* and *L. luteus* kernel meals of the ANF present in soybean meals that induces distal enteritis. In a study examining the intestinal histology of salmon fed a range of diets, including soybean and *L. angustifolius* and *L. luteus* kernel meals a significantly lower level of intestinal damage was observed from the two lupin treatments compared to the soybean treatment. *L. luteus* in particular produced a markedly lower level of intestinal damage, even less than that of *L. angustifolius*. The cause of this intestinal enteritis in salmon occurs predominantly in the sea-water phase of production and is reputed to be caused by the saponin content of soybeans (Knudsen et al., 2006). Interestingly, saponin levels reported in *L. luteus* are about one tenth that of *L. angustifolius,* at 55 mg/kg. The levels of saponins in lupins are generally about one-tenth the amount of that of soybeans, and about half that observed in field peas.

In general the relative lack of ANF in lupins is one of their strong positive features.

End-Note

Much of the information in this section is distilled from the report "Feeding Lupins to Fish: A review of the nutritional and biological value of lupins in aquaculture feeds" available at: http://www.fish.wa.gov.au/docs/op/op031

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Soybean in Aquafeeds

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Introduction

The soybean (*Glycine max*), which is a species within the leguminous bean and pea family Fabaceae, typically the seed contains 20% oil and 40% protein (Table 1). The soybean undoubtedly originated in the orient, probably in China. It was introduced to the USA in the early 1800's as a specialty crop grown in a few botanical gardens. It was not grown as a commercial crop until the 1930's, when increased demand for vegetable oil and the introduction of industrial solvent extraction made processing of soybeans practical.

Today this crop is the world's foremost provider of protein and oil, and from 2000 to 2004 the annual world-wide production of soybeans increased from 160 to 200 million tons (FAO statistics). Being high in lysine and arginine, soybean protein has a favourable amino acid profile for food and feed uses. Consequently, soybean meal (SBM) is one of the most commonly used protein sources in animal feeds. Protein concentrates derived from soybean meals are also important ingredients in various milk replacers and weaning diets.

Soybean protein is also used in aquafeeds, and often with good results. However, soybeans are unusually rich in potent antinutritional factors (ANF), which are the plant's inherent chemical defence against being eaten by herbivores. Consequently these ANF disturb the digestion and/or physiology of animals. This offer significant nutritional and feed technological challenges when soy products represent a major source of dietary protein in diets for carnivorous fish species (Francis et al., 2001).

Table 1. Typical composition of fish meal and soybean protein ingredients (% of DM).

Protein source	Protein	Oil	Starch	Dietary fibre	Sugars
Raw and full-fat soybean	42	21	3	18	11
Dehulled and extracted soybean	57	1	3	23	14
Soy protein concentrate	68	1	7	19	2

Derived from Lusas & Riaz (1995) and Bach-Knudsen (1997).

Digestibility of Soybean Meals

Kaushik et al. (1995) evaluated the digestible value of a wide range of soybean meals of various forms when fed to rainbow trout (Table 2). From this study it was shown that all of the soybean meals had very high protein digestibilities. The extrusion of the soybean meals had little influence on their digestible energy value. The comparison of soybean meal and soy flour showed a minor improvement in the digestibilities of both protein and energy. This was concomitant with the decrease in particle size of the ingredient being examined. This was attributed to an increased availability of both the protein and carbohydrate content of the meals being achieved. The concentration of the protein through development of a protein concentrate was also clearly seen to improve the overall value of soybean meals with an increase in digestible energy content without any loss in protein digestibility.

Table 2. Digestibility values of a range of soybean resources fed to rainbow trout. Data derived from Kaushik et al. (1995).

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

Work by Refstie et al. (1999) examined the relative nutritional value of a range of soy protein products when included in diets for Atlantic salmon (*Salmo salar*) on an equivalent protein basis (Table 3). In this study solvent-extracted soybean meal was compared against an oligosaccharide reduced soybean meal, a soy protein concentrate and a soy protein isolate. Significant improvements to organic matter and protein digestibilities were observed with an increased level of processing of the soybean meals. Notably the digestibility estimates presented by Refstie et al. (1999) differ markedly from those of Kaushik et al., (1995) who evaluated some similar meals, but in different salmonid species (Table 2).

Table 3. Digestibility values of a range of soybean resources fed to Atlantic salmon. Data derived from Refstie et al. (1999).

	Soy Protein Isolate	Soy Protein Conc.	OR-SBM	SE Soybean Meal
Organic matter (%)	69.3	66.0	63.4	61.6
Protein (%)	90.9	89.3	86.7	85.5
Fat (%)	96.2	98.0	93.1	85.7
Starch (%)	50.2	59.3	67.1	54.7
Phosphorus (%)	38.2	33.6	33.6	37.7

OR-SBM: Oligosaccharide reduced soybean meal. SE Solvent-extracted.

In a comparative study between rainbow trout and Atlantic salmon, using the same diets and faecal collection methods, it was observed that energy digestibility of the soy products was generally higher than that of the same ingredients by rainbow trout (Table 4; Glencross et al., 2004). However, protein digestibility was generally higher from all products in the rainbow trout, though protein digestibilities were high for all ingredients in both species. This suggested that the reduction in soy NSP, while not having a dramatic effect on the digestibility of protein in the diet, did affect the Atlantic salmon’s ability to digest energy.

Table 4. Digestibility of protein and energy from soybean meal (solvent-extracted), soy protein concentrate and a soy protein isolate when fed to either rainbow trout or Atlantic salmon. Data derived from Glencross et al. (2004).

	SE Soybean Meal	Soy Protein Conc.	Soy Protein Isolate
Rainbow trout			
Protein	99.0	106.9	97.8
Energy	83.3	85.6	93.1
Atlantic salmon			
Protein	94.4	90.1	97.4
Energy	89.0	101.2	117.4

Growth Studies with Soybean Meals

The work of Kaushik et al., (1995) demonstrated that up to 62% of the diet could be comprised of a soy protein concentrate without loss of feed intake or growth by the fish (Figure 1). In contrast, the inclusion of soyflour at 42% of the diet resulted in a deterioration of growth, though food intake was relatively uninfluenced. When included at 24% of the diet the soyflour proved to be an adequate ingredient. It was suggested that at the higher inclusion level of soyflour, that the NSP content of this ingredient was causing problems with nutrient absorption by the fish. In addition the influences of oligosaccharides and saponins were also suggested.

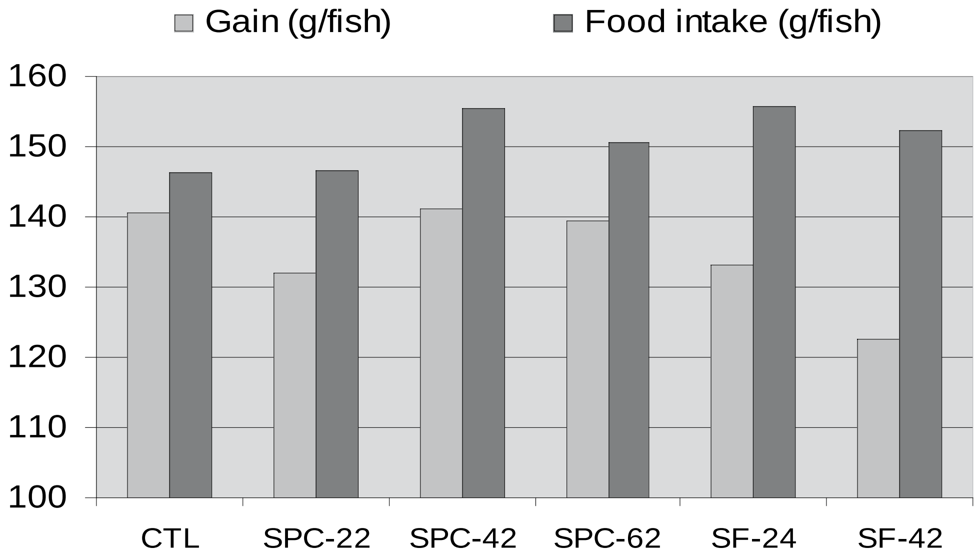

Figure 1. Growth (weight gain) and food intake (g/fish) of rainbow trout fed diets with increasing inclusion levels of soy protein concentrate (SPC at 22%, 42% and 62%) or soy flour (SF at 24% and 42%). CTL is a fishmeal based reference diet. Data are derived from Kaushik et al. (1995).

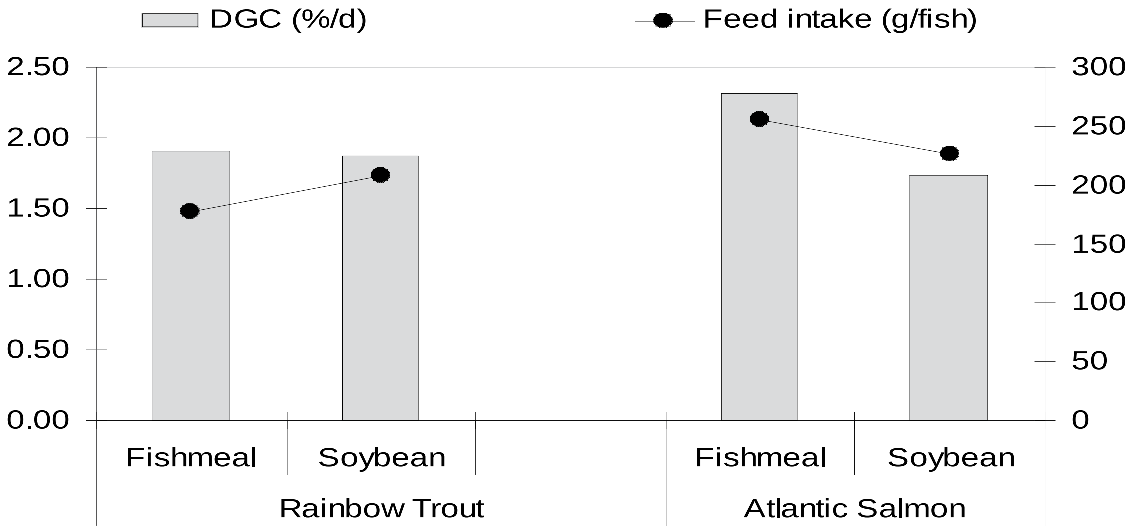

Figure 2. Growth rate (DGC, %/d) and food intake (g/fish) of rainbow trout or Atlantic salmon fed diets with or without soybean meal (30%inclusion). Data are derived from Refstie (2000).

In a growth study designed to compare the responses of rainbow trout and Atlantic salmon when fed soybean meal differing responses were observed between the two fish species (Figure 2; Refstie, 2000). Two diets, one with ~30% soybean meal and the other with only fish meal as its sole protein source were fed to either species for 84-days. After this period, growth by the rainbow trout was similar on both diets, although the feed intake was much higher for the soybean meal diet. In contrast, Atlantic salmon reduced their intake of the soybean meal diet and their growth rate also similarly reduced. Digestibility of diets by either species also differed. While protein digestibility was relatively consistent for each diet within each fish species, there was a significant decline in the digestibility of lipid by the Atlantic salmon on the soybean diet. This decline in lipid digestibility had a small, but notable effect on the energy digestibility of soybean diet when fed to the Atlantic salmon. Both rainbow trout and Atlantic salmon exhibited morphological changes in the distal intestine when fed the soybean meal diet.

Soybean Meals and Gut health in Fish

The fish intestine is a complex multifunctional organ that in addition to digesting feedstuffs and absorbing nutrients is critical for water and electrolyte balance, endocrine regulation of digestion and metabolism, and immunity. Intestinal structure and function in fishes depends on feeding habit as well as different evolutionary processes, and is, thus, highly variable among species. The basic mechanisms of fish digestion are similar to those of mammals, but unlike mammals, fish retain the capacity to absorb macromolecules throughout life. This ability is best developed in the distal intestine, and this intestinal section also has a high density of immune cells.

Some sensitive fish species are apparently intolerant to certain feedstuffs. This has been shown in salmonid (*Salmo* and *Oncorhynchus*) species, which in response to full-fat, and extracted soybean meal (SBM) develop a condition termed soybean meal-induced enteritis, which is most pronounced in the distal intestine (Baeverfjord and Krogdahl, 1996). This condition is also induced in salmonids by the alcohol-extract (velasse) resulting from alcohol washing of SBM to produce soy protein concentrate (Ingh et al., 1991, 1996) but not when feeding the resulting soy protein concentrate (SPC).

The morphological changes and inflammation in the distal intestine of salmonids (Figure 3) have been characterised as followed: Shortening of the primary and secondary mucosal folds with a widening of the central stroma (*lamina propria*) and submucosa, shortened microvilli of the brush border membrane and increased formation of microvillar vesicles, and a dramatic decrease or even absence of the normal supranuclear absorptive vacuoles in the enterocytes (Baeverfjord and Krogdahl, 1996). The lamina propria is widened with a profound infiltration of a mixed population of inflammatory cells such as lymphocytes, neutrophilic granulocytes, cells of monocytic lineage, including macrophages, eosinophilic granular cells, and diffuse IgM (Baeverfjord and Krogdahl, 1996; Bakke-McKellep et al., 2000).

Due to the infiltration of inflammatory cells and rapid regression of the condition following withdrawal of soybean meal from the diet, the condition has been classified as a non-infectious, subacute enteritis (Baeverfjord and Krogdahl, 1996), suggesting an etiology involving immunological mechanisms. The pathogenesis may involve immunological mechanisms similar to that of a hypersensitivity reaction (Baeverfjord and Krogdahl, 1996), although plasma of rainbow trout fed SBM-containing diets is negative for specific antibodies against soy protein (Kaushik et al., 1995) despite increased general immunoglobulin levels. Increased number of proliferating cells lining the villous folds of the distal intestine of soybean meal fed salmon (Sanden et al., 2005) suggests disturbed functionality of enterocytes due to alterations in enterocyte turnover and degree of maturation.

In terms of reduced digestive capacity, these morphological changes reduce the mass of the distal intestine (Nordrum et al., 2000; Refstie et al., 2006ac). The digestive process is also altered. The activity of brush border membrane bound (Bakke-McKellep et al., 2000; Krogdahl et al., 2003; Refstie et al., 2006ac) and cytosolic (Bakke-McKellep et al., 2000) digestive enzymes in the distal enterocytes is reduced, and the carrier-mediated transport of amino acids and glucose is lowered while the permeability of distal intestinal epithelium for nutrient transport increases (Nordrum et al., 2000). The absorption of macromolecules by the distal intestine furthermore decreases (Bakke-McKellep, 1999), apparently causing reduced reabsorption of endogenous digestive secretions, as indicated by dramatically increased activity of trypsin in the distal intestinal contents (Krogdahl et al., 2003; Refstie et al., 2006ac). The expression of soybean meal-enteritis is dose dependent (Krogdahl et al., 2003), but it is clearly expressed when feeding <10% SBM.

How much the condition actually contributes to reduced nutrient absorption is unclear, however, as the distal intestine is not recognised as a major absorptive site in fish (Refstie et al., 2006c). Furthermore, salmonids suffering the condition appear to grow normally (Refstie et al., 2000; 2001; 2005). They do, however, appear more susceptible to infectional diseases (Krogdahl et al., 2000)

The antigen(s) inducing this inflammatory response are still not identified. It is also unclear whether other plant seeds may have similar antigenic properties. The antigen(s) are apparently soluble in alcohol, as alcohol washed soy protein concentrates do not induce enteritis, whereas the alcohol extract (soy velasse) does (Ingh et al., 1996; Krogdahl et al., 2000).

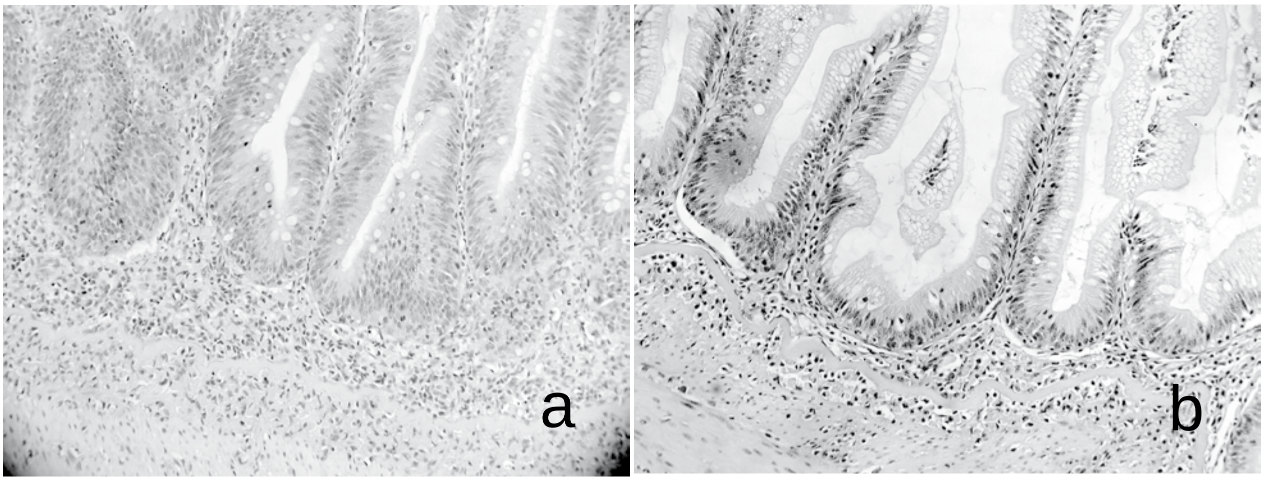

Figure 3. Normal distal intestine of Atlantic salmon fed fish meal (a), compared to typical soybean meal-induced morphological changes in the distal intestine of salmon fed 40% SBM (b).

Processing and Nutritive Value of Soybean Proteins in Fish

Soybeans are bred to contain lower levels of, and less potent proteinase inhibitors and agglutinating lectins. Noteworthy, there already exist soy cultivars with low inherent levels of these antinutritional factors (Table 5). Such criteria are important when selecting plant seeds for salmonid diets. However, the availability of such soy cultivars is still limited.

The processing routes of conventional soybeans to SBMs and soybean protein concentrates (SPC) are outlined in Figure 4. Moderate heating of soybeans reduces the soy TI- and lectin activities to levels tolerated by salmonids (Anderson and Wolf, 1995; Figure 4). Thermal denaturation and inactivation of TI and agglutinating lectins requires moist heat. Defatted soy flakes are typically toasted (steam-cooked) at 105 °C for 30 minutes to remove solvent residues after the oil extraction procedure (Lusas and Riaz, 1995).

As Table 5 shows, this reduces the TI-activity down to levels tolerable by salmonids. It is also paralleled by denaturation and inactivation of the lectins. Furthermore, soybean meals in modern aquafeeds are subjected to a second moist heating during high-pressure moist extrusion manufacturing of the diets. Thus, TI and lectins are rarely problematic when using extracted soybean meal in salmonid feeds. They may, however, cause problems when producing feeds from insufficiently toasted full-fat soy and certain unheated beans.

It follows that the availability of nutrients in SBM highly depends on how well the thermal treatment of the meal is optimised. Insufficient thermal sum leaves active TI and lectins, while too high thermal sum induced heat damage on the protein.

Soybean phytosterols, saponins, and agents(s) inducing distal enteritis in sensitive fish species are all soluble in alcohol. Thus, such ANF factors are removed by alcohol and water washing when manufacturing SPC. This leaves phytic acid, which should be hydrolysed if vegetable feed ingredients accounts for a major part of the dietary protein.

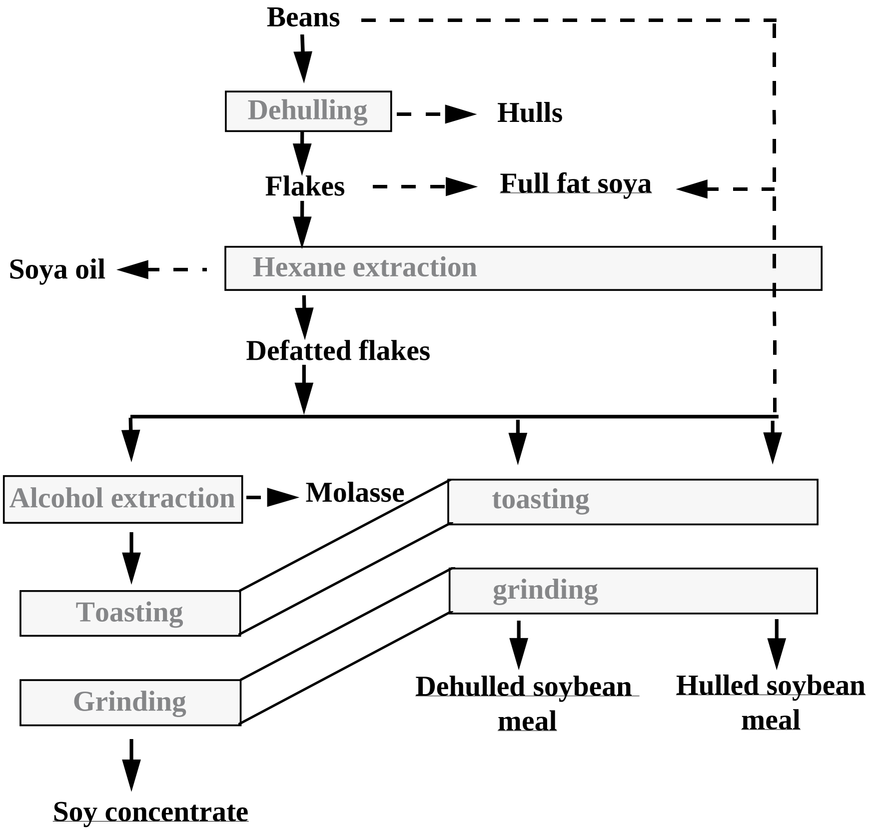

Figure 4. Schematic flow diagram of the major processing routes for soybeans used in fish feed.

Soybean Antinutritional Factors

Vegetable ANF may broadly be divided into heat-stable and heat-labile factors. Unless intolerable levels of these components can be inactivated or removed, they constitute the major restrictor on use of vegetable protein in feeds. As the term implies, heat-labile factors may be destroyed or inactivated by thermal processing. Soybeans, are rich in heat-labile antinutritional factors, such as proteinase inhibitors and agglutinating lectins (Table 5). Proteinase inhibitors are proteins capable of binding protein-hydrolysing digestive enzymes, thus restricting digestion and utilisation of dietary protein. Salmonid intestinal proteinases have high substrate affinity, and are more sensitive to proteinase inhibitors than proteinases of warm-blooded animals and most investigated fish species. Lectins are glycoproteins that bind (agglutinate) to receptors in the epithelium of the salmonid intestine, possibly with deleterious effects.

Proteinase inhibitor-activity is commonly measured as mg bovine trypsin inhibited per g sample (TI-activity). TI-activity may exceed 30-mg/g in raw soybeans. As shown by Figure 5, both nutrient digestibility and growth by Atlantic salmon (*Salmo salar*) are severely reduced if the dietary TI-activity exceeds 5 mg/g. This is similar in rainbow trout (*Oncorhynchus mykiss*; Krogdahl et al., 1994), and probably salmonids in general.

Table 5. Contents of Kunitz trypsin inhibitor (TI), Bowman-Birk combined trypsin and chymotrypsin inhibitor (BB-TI), and agglutinating lectins, together with activities of functional TIs, Lectins, and urease in raw and processed legumes.

Protein source	Contents of			Activities of		
	Kunitz-TI mg/g	BB-TI mg/g	Lectins g/g	TIs mg/g*	Lectins mg/g**	Urease pH rise***
Raw soybean						
Conventional[1,2,3,4,5]	30.30	10.7	8.3	17 – 31	2.3	2.1
Kunitz inhibitor-free[2,3]	0.04	11.4	8.0	5.6		2.0
Lectin-free[3]	28.40	13.0	<0.0002			
Toasted soybean meal[2,4,5,6,7]			3 – 9	0.01 – 0.2	<0.2	
Soy protein concentrate[4,6]			2 – 7			

*mg bovine trypsin inhibited per g meal.

**mg lectins agglutinating to brush border vesicles of chicken per g meal.

***pH rise in phosphate buffer as urease acts upon urea to produce ammonia.

Derived from Pisulewska & Pisulewski, 2000; Dhurandhar & Chang, 1990; Han et al., 1991; Douglas et al., 1999; Anderson & Wolf, 1995; Maenz et al., 1999; Refstie et al., 1999; Waldroup et al., 1985.

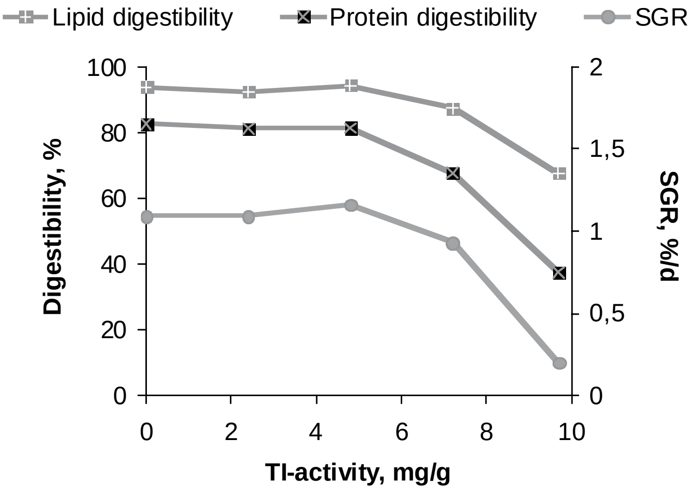

Figure 5. Reduced apparent digestibility of protein and lipid, and lowered specific growth rate (SGR) in Atlantic salmon fed diets with increasing trypsin inhibitor (TI) activity. Data are extracted from Olli et al. (1994).

The most significant heat-stabile soybean ANF are phytic acid, which restrict absorption of divalent ions (Francis et al., 2001), and agents that induce morphological changes and inflammation in the intestinal mucosa of some fish species (SBM meal-induced enteritis; described by Baeverfjord and Krogdahl, 1996). In salmonid species, meal-induced enteritis in the distal intestine is induced by dietary full-fat as well as solvent-extracted SBM, and also the alcohol-extract (velasse) resulting from alcohol washing of SBM to produce soy protein concentrate (van den Ingh et al., 1991, 1996). Saponins and phytosteroles may also be problematic, although these components are little studied in fish (Francis et al., 2001).

Dietary soybean meals cause reduced digestibility of lipid when substituting fish meal in the feed. This reduced digestibility of lipid is not caused by the lipid moiety of the soy, since it is pronounced when using diets with solvent extracted soybean meal. The soybean agent(s) causing it are not identified, although saponins, phytosterols and/or dysfunctional distal intestine due to SBM-induced enteritis are suspected. Reduction in the lipid digestibility of more than 10% have been reported when replacing 35% of fish meal protein with extracted soybean meal in feed for Atlantic salmon (Refstie, 2000). Highly processed soy products, however, cause little or no reduction in fat absorption in salmon. This reduced uptake of dietary lipids has consequences both for the net energy value of the feed, and probably also for utilization of lipid-soluble nutrients. Thus, calculated values for digestible or metabolisable energy in diets with soy meal, based on chemical composition and constant factors, are not valid unless corrected for this non-additive factor. Heat labile ANF are hard to inactivate, and, thus, restrict the use of soybean proteins in aquafeeds if not removed. From this it follows that the nutritional value of soybean protein to fish depends on proper processing of the soybeans, and that the need for processing and refining varies among species.

Future

To conclude, most ANF in soybeans are removed by thermal treatments followed by washing to produce SPC. However, these are elaborate treatments to produce high-priced products. Hence, it is important to focus on eliminating critical ANF by cost-effective technological means and/or breeding of seed cultivars with low inherent levels of the ANF. Possible antigenic factors inducing enteritis in sensitive fish species must be identified and eliminated. It is also desirable to eliminate non-starch polysaccharides in order to enhance the digestibility of energy.

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Field Peas and Aquafeeds

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Introduction

Field peas are the harvested seed of species from the *Pisum* genus, a group within the leguminous bean and pea family Fabaceae. Field peas (*Pisum sativum*) are another ingredient with nutritional potential for aquaculture feeds, which Australia also has some capacity to supply. Production of peas in Australia as a legume is second only to that of lupins, at a production level of around 371,000 tonnes per annum. The majority of the field pea production in Australia occurs in South Australia and Victoria.

Compositional Features of Pea Meals

Field peas, although legumes from the same family as lupins and soybeans differ considerably in their composition. Notably pea meals have a lower protein content (~25 %), a very low fat content (~1%) and a high carbohydrate content (~70%), which is predominantly starch. It is this predominance of starch that makes peas quite a different prospect than that of lupins and soybean (Table 1). The main limitation to use of pea meals in aquaculture feeds is the lower protein level, which makes it generally uncompetitive against other grain varieties such as soybean and lupins. However, its combined starch and protein content does lend itself to co-processing and development of value-added products.

Table 1. Gross chemical composition of Field pea meals and other grain varieties.

Grain	DM	Protein	Fat	Ash	Starch	NSP
Soybean						
Solevent-extracted	89	48	1	9	1	31
Full-fat	91	42	20	5	1	24
Lupin						
L. angustifolius seed	91	32	6	3	1	50
L. angustifolius kernel	90	39	7	3	1	41
L. albus kernel	92	44	11	4	1	33
L. luteus kernel	90	52	7	4	1	27
L. mutabilis kernel	91	52	17	4	1	18
Field Pea						
Whole seed	90	23	1	3	32	33
Kernel meal	91	26	2	2	45	17

Digestibility of Peas

The digestible value of field pea meal has been compared against a variety of other feed grain meals when fed to rainbow trout (Table 2; Gomes et al., 1995). Generally field pea meal had apparent dry matter digestibility similar to that of the lupin meal, but lower than that of soybean meals. The protein digestibility of the field pea meal was also somewhat lower than that of the other plant protein meals. The energy digestibility of the field pea meal was also one of the lowest reported. It should be noted that in this study the peas or lupins were whole-seed meals.

Table 2. Digestibility values of pea seed meal compared to a range of feed grain resources, when fed to rainbow trout. Data derived from Gomes et al. (1995).

	Dry matter digestibility (%)	**Protein digestibility (%)**	**Energy digestibility (%)**
Fishmeal	78.0	86.6	69.7
Lupin *(L. angustifolius)* whole-seed meal	63.3	85.5	61.2
Full-fat toasted soybean meal	75.4	86.4	80.2
Full-fat micronised soybean meal	86.6	96.3	90.7
Faba bean meal	66.1	80.2	60.2
Pea seed meal	66.6	80.4	59.2
Maize gluten	90.7	95.3	91.8
Co-extruded pea and canola meal	89.6	94.5	87.2
Meat meal	94.1	90.8	92.1

Burel et al. (2000) examined the value of extruded peas, extruded *L. albus* kernels and two different rapeseed meals. Key findings were the significantly lower protein digestibility of the pea meal in comparison to the *L. albus* kernel, but not the rapeseed meals. In addition, the energy digestibility of pea meal was also significantly less than that of the *L. albus* kernel meal, though not that of either of the rapeseed meals. In most cases, the relative digestibility of the energy of each of the ingredients was a direct response to the protein content of the ingredient and the relative protein digestibility of that ingredient. Although the levels of starch in the pea meals would have contributed to their digestible dietary energy content, given that the level of protein in the meal is substantially less than the other meals, the energy digestibilities are generally similar.

In a study undertaken by Carter and Hauler (1999), the digestible value of diets including a pea meal were compared against diets including soybean meal or *L. angustifolius* (cv. Gungurru) kernel meal, when fed to Atlantic salmon. From this study the highest apparent protein (nitrogen) digestibilities were those observed from the diets which included the pea meal. However there were no significant differences in protein digestibility observed among any of the three grain varieties. Apparent energy digestibilities were highest from salmon fed the soybean diet. Second highest were from those fed the pea meal.

Table 3. Proximal composition and nutritional value of various plant meals to fed rainbow trout. Data derived from Burel et al. (2000).

	Extruded peas	Extruded *L. albus*	SE-Rapeseed	HT-Rapeseed
Ingredient Proximate Composition				
Dry matter (g/kg)	909.0	928.0	937.0	915.0
Crude protein (g/kg DM)	260.0	434.0	431.0	433.0
Crude fat (g/kg DM)	45.0	100.0	48.0	9.0
Ash (g/kg DM)	33.0	46.0	79.0	82.0
NFE (g/kg DM)	612.0	348.0	379.0	391.0
Phosphorus (g/kg DM)	4.4	5.4	14.9	15.6
Nutrient Apparent Digestibility				
Dry matter (%)	66.3	69.7	70.8	66.6
Protein (%)	87.9	96.2	90.9	88.5
Energy (%)	68.9	77.0	76.4	70.0
Phosphorus (%)	42.6	61.9	26.4	41.8

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

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

Booth et al., (2001) also examined the nutritional value of a range of other plant legumes including field peas, faba beans, chick peas and vetch when fed to silver perch (Table 4). In this study each of the grains was also evaluated in whole-seed meal and kernel meal forms. The dehulling process realised only a minor increase in the protein content of the pea meal from about 25.5% to 27.7%. The protein digestibility of the field pea meals was good and was exceeded only by that of the lupin meals and the Faba bean meals. Improvements in protein digestibility of field peas were observed with dehulling. This effect was consistent across all of the legume meals examined, excepting chickpea meals. Energy digestibilities of each of the plant legumes were improved with dehulling of the grains. Although digestibility of the Faba bean meals was an exception to this with a moderate decrease in energy digestibility with dehulling. Notably the carbohydrate content of each the meals examined is predominated by starch. Therefore it is likely that the fish are obtaining some energetic value of from the starch content of some of these grains. This contrasts the observations of utilisation of the carbohydrate fraction of soybeans and lupins, which are dominated by non-starch polysaccharides. Dry matter digestibilities of field peas were considerably improved with the removal of the seed coat (dehulling). This was a similar finding to that observed with lupins.

In a subsequent study, Allan and Booth (2004) examined the influence of dehulling and extrusion on the digestible value of a range of feed grains, including Field peas (Table 5). It was again observed that dehulling significantly improved the digestible value of dry matter, protein and energy for field peas. Extrusion of the field peas did have a benefit with slight improvements observed in both the protein and energy digestibilities.

Table 4. Proximal composition and digestibility value of various plant meals to fed Silver perch. Data derived from Booth et al. (2001).

	Ingredient Proximate Specifications (g/kg)				Ingredient Digestibilities		
	Protein	Fibre	Energy (MJ)	Fat	Dry matter (%)	Protein (%)	Energy (%)
L.angustifolius whole-seed	341	-	17.9	57	50.3	96.6	59.4
L.angustifolius kernel meal	436	-	20.7	6.6	67.6	100.3	74.0
Field pea whole-seed	255	87	17.0	11	48.9	83.3	54.5
Field pea kernel-meal	277	28	17.3	10	62.0	88.1	67.0
Faba bean whole-seed	277	120	17.3	13	55.9	91.6	62.2
Faba bean kernel meal	313	33	17.6	13	58.2	96.6	58.8
Chickpea whole-seed	208	134	19.4	47	48.7	84.8	53.6
Chickpea kernel meal	242	25	19.3	50	58.4	81.2	60.2
Vetch whole-seed	309	72	17.9	9	41.5	74.9	55.5
Vetch kernel meal	323	41	18.6	9	78.3	87.7	81.8

Table 5. Proximal composition and digestibility value of pea meals to fed Silver perch after being subjected to a range of processing methods. Data derived from Booth et al. (2001).

	Field pea - *P. sativum*			
	whole-seed raw	**kernel raw**	**whole-seed extruded**	**kernel extruded**
Ingredient composition				
Protein (g/kg DM)	258.0	262.0	257.0	274.0
Fat (g/kg DM)	12.0	16.0	5.0	7.0
Ash (g/kg DM)	33.0	28.0	34.0	28.0
Gross energy (MJ/kg DM)	18.1	18.5	18.4	18.5
Phosphorus (g/kg DM)	5.0	4.0	5.0	4.0
Ingredient digestibilities				
Dry matter (%)	56.4	69.3	69.6	77.7
Nitrogen (%)	81.8	90.3	84.2	96.4
Energy (%)	56.1	69.0	70.8	78.7

Growth Studies with Pea Meals

Gouveia and Davies, (1998) carried out a preliminary evaluation of pea seed meal in diets for European sea bass. The pea seed meal was prepared from whole peas by dry cooking followed by grinding. In a growth study it was demonstrated that up to 40% pea seed meal inclusion (the maximum level tested) could replace 12% of the fish meal in the diet without impairment to growth performance or nutrient retention. In a subsequent study the same authors undertook a growth trial with groups of juvenile European sea bass fed diets containing 0, 10, 20 and 30% of a commercial pea seed meal (Gouveia and Davies, 2000). The pea meal in this instance was highly processed as a dehulled, defibered, destructured, cooked, sterilized and micro ground product. In this study there was no indication of negative effects in the growth trial at any inclusion level (Figure 1).

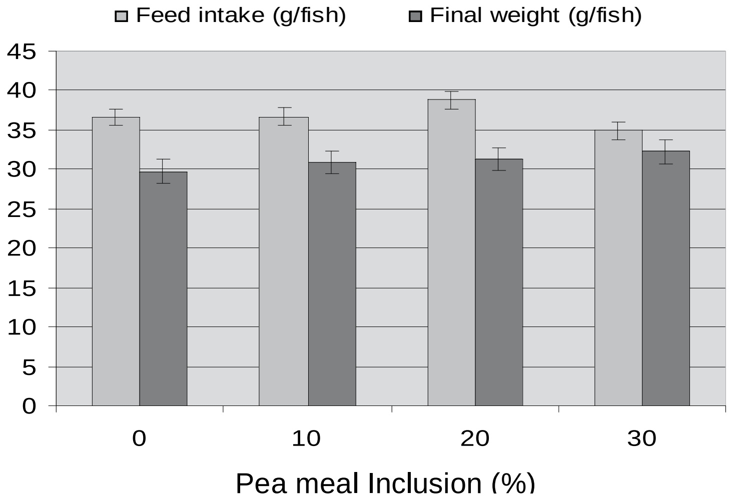

Figure 1. Final weight and feed intake by European seabass fed diets containing increasing levels of dehulled extruded pea meal.

Carter and Hauler (2000) evaluated the nutritional value of diets containing a pea protein concentrate and compared the nutritional value of this processed form of the grain relative to that of defatted soybean meal and a *L. angustifolius* (cv. Gungurru) protein concentrate. Both protein concentrates were produced using air-classification methods and had protein levels less than 50%. The three protein resources were included in diets at either 25% or 33% replacement of the fishmeal protein content of the diet (Figure 2). Assessment of the nutritional value of the diets fed to the juvenile Atlantic salmon in Carter and Hauler's (2000) study supported that each diet maintained growth equal to that of the control/reference diet (Figure 2). Food consumption was significantly higher by fish fed the diet in which the fishmeal was replaced by 29% *L. angustifolius* protein concentrate inclusion. As a consequence of this, the food conversion of fish fed this treatment was also significantly poorer than the other treatments in the study. The diet with 21% inclusion of pea protein concentrate resulted in the best growth and feed conversion, with no difference in feed intake observed with higher inclusion levels of the pea protein concentrate. Based on the results from this study, Carter and Hauler (2000) supported that extruded Atlantic salmon feeds could reasonably contain in excess of 27% of pea protein concentrate. It was also suggested that it would be more likely that such plant protein resources be used in combinations, rather than as single protein meals.

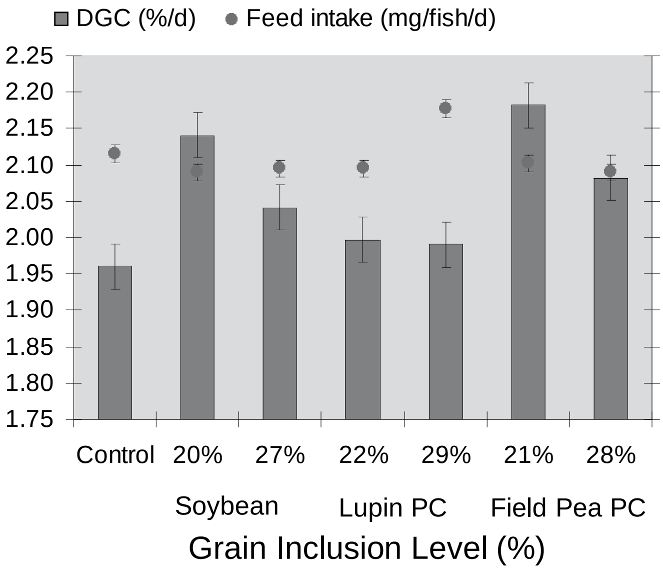

Figure 2. Growth rate and feed intake by Atlantic salmon fed diets containing soybean, lupin protein concentrate or pea protein concentrates.

A feeding and digestibility trial was undertaken with juvenile Coho salmon (*Oncorhynchus kisutch*) to determine the nutritional value of differently processed pea ingredients (He et al., 2002; Figure 3) as partial or total replacement of fishmeal protein. The diets were formulated on an isonitrogenous and isolipidic basis (48% crude protein and 19% lipid). The results from this study showed that micronized pea and dehulled extruded pea could replace all the wheat and some of the fishmeal protein. However, the limiting factor to dietary inclusion of these ingredients was their inherent low protein levels. In contrast, pea protein concentrate was well accepted and utilised at low inclusion levels, but above 15% inclusion a substantial deterioration in diet digestible energy value was noted. Growth however was maintained but the poorer digestible energy level led to an increase in the feed intake and a subsequent deterioration in the feed efficiency (Figure 3).

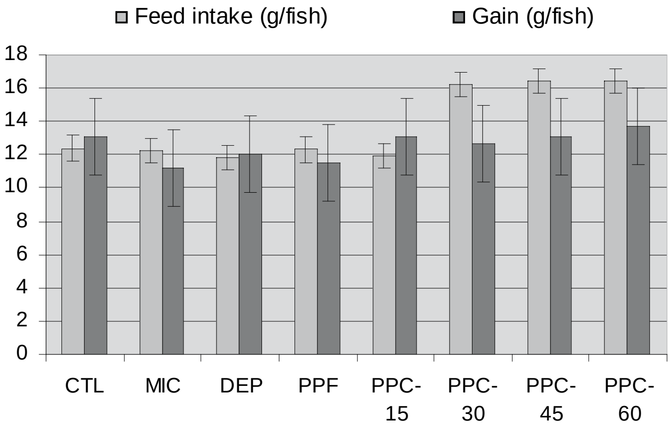

Figure 3. Growth (weight gain) and feed intake by Coho salmon fed diets containing micronised pea meal (MIC), dehulled extruded pea meal (DEP), pea protein flour (PPF) or different inclusion levels of a pea protein concentrate (PPC15% to 60%) compared to a fishmeal control diet (CTL).

Various agricultural ingredients were evaluated for their potential to reduce the amount of fishmeal used in feeds for silver perch (Booth and Allan, 2003). A growth experiment was conducted to evaluate different inclusion levels of dehulled field peas, peanut meal, canola meal and a meat meal. The results indicated that silver perch grew better on diets containing up to 45% each of peanut meal, meat meal, canola meal or field peas compared to silver perch fed similar quantities of a non-nutritive filler. This simple comparison demonstrated that silver perch were able to use these ingredients to support weight gain. According to the modelled data, silver perch were inefficient at utilising the digestible protein from diets containing more than 75% field peas. In terms of digestible energy, silver perch were inefficient at utilising diets, which contained 75% field peas and 75% peanut meal.

Anti-Nutritional Factors in Pea Meals

A variety of anti-nutritional factors (ANF) are found in pea meals (Table 2). Pea meals in particular are noted for their tannin and protease inhibitor content. Tannins are a group of polyphenolic compounds that bind to proteins to either inhibit their activity in the case of digestive enzymes or to prevent their digestion, in the case of most other proteins. Tannins can also form cross-linkages between proteins and other macro-molecules and render them unavailable for digestion. These inhibitory facets, in conjunction with an astringent taste constitute the anti-nutritional characteristics of tannins (Petterson, 2000). Protease inhibitors are molecules present in the grain that restrict the activity of proteolytic enzymes like trypsin, thereby reducing the protein digestion that occurs in the gut of the animal.

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

Table 2. Anti-Nutritional Factors (ANF) found in key feed grain varieties.

All values mg/kg DM	Alkaloids	Phytate	Tannins	Trypisin Inhibitor	Polyphenolics	Oligosaccharides
L. angustifolius cv Mandelup KM	33	5,222	0	9,222	2,889	85,556
L. angustifolius cv Myallie KM	43	4,839	0	5,161	2,796	62,366
L. angustifolius cv Merrit KM	11	5,761	0	1,957	2,391	64,130
L. angustifolius cv Belara KM	11	5,889	0	6,444	2,778	88,889
L. luteus cv Wodjil KM	143	7,253	0	2,857	2,418	102,198
L. albus cv Kiev mutant KM	23,182	4,545	568	6,250	15,341	78,409
Whole Soybean	292	8,202	0	22,247	4,719	40,449
Solvent Extracted Soybean meal	44	8,889	0	10,000	4,556	62,222
Solvent Extracted Canola Meal	22	12,043	1,505	10,860	15,269	11,828
Whole Canola (Surpass 501TT)	22	10,440	549	9,451	14,835	12,088
Whole Field Pea (Laura-Dunwa)	11	5,435	7,826	8,152	9,565	33,696
Dehulled Field Pea (Laura-Dunwa)	11	6,154	989	10,989	2,527	36,264

End-Note

A detailed on-line review on the use of pea products in aquaculture feeds is" The Use of Peas in Aquafeeds" available at: http://www.infoharvest.ca/pcd/summ2004/sect05.html

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Canola/ Rapeseed in Aquafeeds

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Introduction

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

Canola is grown throughout the Australian wheat-belt, with the majority of the grain produced in Western Australia, New South Wales, South Australia and Victoria. About 1,650,000 tonnes of canola is produced in Australia annually worth an estimated $400 million (2000-2005 averages; ABARE, 2005). The majority of this is exported as whole-seed, though there is a significant volume of domestic oil extraction producing both canola oils and meals.

Compositional Features of Lupin Meals

Two main processes are currently used in Australia to extract oil from canola seed - solvent extraction and expeller extraction. A third process, cold pressing is also used in some cases, though volume of production through this processing method is not large. These processes extract oil with varying efficiency, and as a result the total protein, fat content and gross energy content of the resulting meals can vary.

Table 1. Gross chemical composition of canola meals and other grain varieties.

Grain	DM	Protein	Fat	Ash	Starch	NSP
Soybean						
Solevent-extracted	89	48	1	9	1	31
Full-fat	91	42	20	5	1	24
Lupin						
L. angustifolius seed	91	32	6	3	1	50
L. angustifolius kernel	90	39	7	3	1	41
L. albus kernel	92	44	11	4	1	33
L. luteus kernel	90	52	7	4	1	27
L. mutabilis kernel	91	52	17	4	1	18
Rapeseed						
Expellet-extracted	90	34	12	4	1	39
Solevent-extracted	90	39	2	6	1	43

Protein content in solvent-extracted canola meal is generally higher than that of expeller-extracted canola meals. In contrast the residual fat remaining in expeller-extracted meals, which accounts for its comparatively lower protein, also provides a higher meal fat level and therefore a greater energy content in the meal. The carbohydrate content of canola meals is largely devoid of starch.

Protein quality of canola meal is affected by the various processing steps involved in their production. The processes of seed conditioning, expelling, mechanical extrusion and solvent extraction can all damage protein quality. However, the extent of this damage can be determined by assessing a range of factors including the heating temperature and time exposed at that temperature. Notably, excess heating during these oil extraction processes can result in reduced digestibility of protein

Nutritionally, the composition of canola meal is quite favorable as an aquaculture feed ingredient. It has protein levels similar to that of other plant meals and its amino acid profile is particularly well suited to use in aquaculture diets. Digestibility of most nutrients seems to be similar to that of other plant protein meals (Burel et al., 2000a). However, some limitations have been suggested to the maximum inclusion levels of this ingredient (Higgs et al., 1983; Burel et al., 2000). This is primarily due to the levels and types of anti-nutritional factors (ANF) present in the seed, notably glucosinolates and associated breakdown products, as well as phytates, cellulose and the total fibre content.

Digestibility of Canola Meals

The nutritional value both solvent and heat-treated European rapeseed meals and also that of extruded *L. albus* kernel meal and extruded peas has been examined (Table 1; Burel et al., 2000). From this work the digestibility characteristics of the dry matter, protein, energy and phosphorus contents of each of the rapeseed meals were defined. Key findings from the work were the significantly higher protein digestibility of *L. albus* kernel meal in comparison to the pea and the two rapeseed meals. However, despite having relatively poor protein/nitrogen digestibility, the energy digestibility of the solvent-extracted rapeseed meal was as good as that of the *L. albus* kernel meal. Notably, the heat-treated rapeseed meal had poorer energy digestibility. This is probably a direct reflection of the markedly lower fat content (43 g/kg DM cf. 9 g/kg DM) of the heat-treated meal.

The digestibility of both solvent-extracted and expeller-extracted canola meals has been assessed relative to a canola protein concentrate and solvent-extracted soybean meal (Figure 1; Glencross et al., 2004a). In this assessment the digestibility of protein, energy, organic matter and phosphorus were determined. Protein (N) digestibility in expeller-extracted meal was significantly better than that of the canola protein concentrate and the soybean meal, but only marginally better than that of the solvent-extracted meal. Energy digestibility of the solvent-extracted meal was significantly poorer than all of the other meals, while there was no significant difference between the energy digestibility of the soybean meal and the expeller-extracted canola meal. The canola protein concentrate had a significantly higher energy digestibility than all of the other meals, most likely reflective of its higher protein content and reduced level of indigestible carbohydrates.

Table 2. Proximal composition and nutritional value of various plant meals to fed rainbow trout. Data derived from Burel et al. (2000).

	Extruded peas	Extruded Lupin	SE-Rapeseed	HT-Rapeseed
Ingredient Proximate Composition				
Dry matter (g/kg)	909.0	928.0	937.0	915.0
Crude protein (g/kg DM)	260.0	434.0	431.0	433.0
Crude fat (g/kg DM)	4.5	100.0	48.0	9.0
Ash (g/kg DM)	33.0	46.0	79.0	82.0
NFE (g/kg DM)	612.0	348.0	379.0	391.0
Phosphorus (g/kg DM)	4.4	5.4	14.9	15.6
Nutrient Apparent Digestibility				
Dry matter (%)	66.3	69.7	70.8	66.6
Protein (%)	87.9	96.2	90.9	88.5
Energy (%)	68.9	77.0	76.4	70.0
Phosphorus (%)	42.6	61.9	26.4	41.8

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

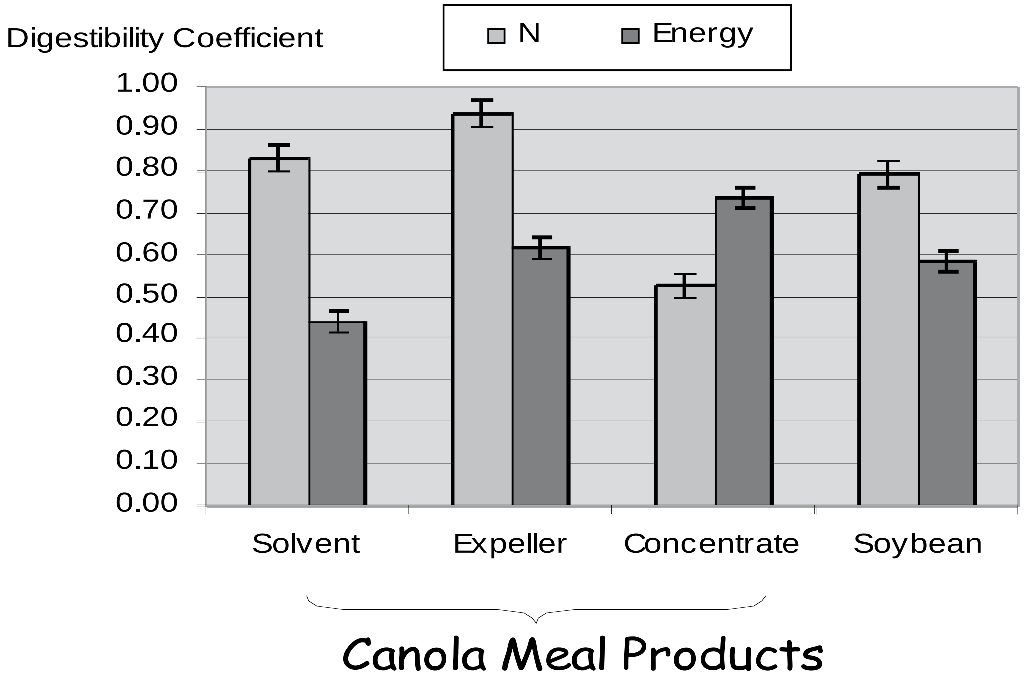

Figure 1. Protein (N) and energy digestibilities of solvent-extracted and expeller-extracted canola meals and a canola protein concentrate, compared to solvent-extracted soybean meal.

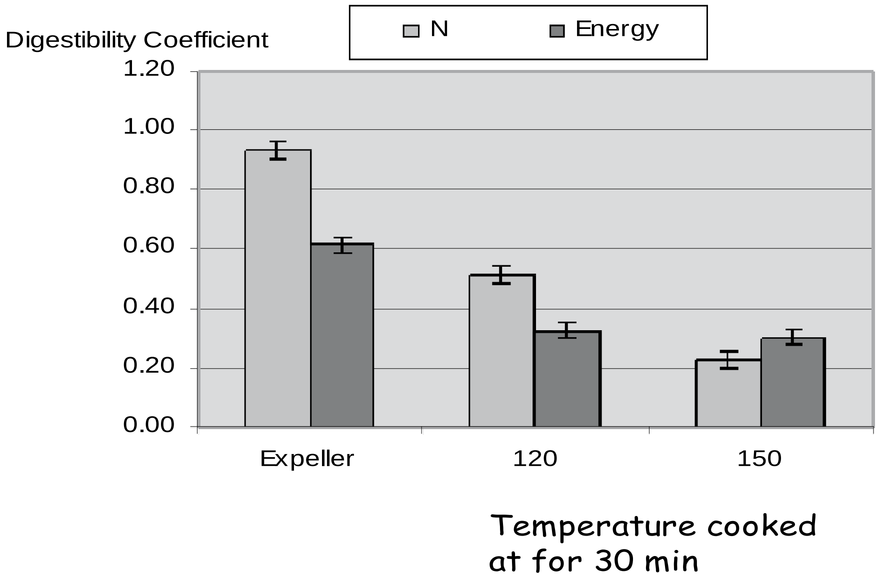

Figure 2. Effect of heat on the digestibility of canola meals.

The influence of heat was also shown to have considerable negative impacts on the nutritional value of canola meals (Figure 2; Glencross et al., 2004a). This was seen primarily as a reduction in the levels of digestible protein and energy in the meals. The implications of these findings are quite clear, in that processors need to be aware of the sensitivity of fish to heat damage in protein resources. However, the distinct nature of this heat damage, whether it is cumulative heat or critical temperature, that is important is not known.

Growth Studies with Canola Meals

The effects of European rapeseed meals on growth of rainbow trout were examined by Burel et al. (2001). In this study the two rapeseed meals contained glucosinolates at concentrations of either 26 mmol/kg or 40 mmol/kg. Each of the rapeseed meals was included in diets at incremented levels of 30% and 50% (Figure 3). This work claimed that the influence of the rapeseed meal glucosinolates on the thyroid axis of fish was sufficient to induce problems with the dietary inclusion of this meal. This was clearly supported by the reduced growth and feed intake by the fish and worsening FCR's with increasing dietary glucosinlate levels (Figure 7). Some aberrations to the thyroid hormone metabolism of the fish were also noted.

However studies by Higgs et al. (1982; 1983) provided support that Canadian canola meals could be useful ingredients when included in diets for fish. Studies examining incremental inclusion levels of a canola meal observed declining growth (Figure 4). This work also identified that glucosinolates were a potential problem with high inclusion levels, but that there was some potential for aversion of this by using dietary additives such as the hormone tri-iodothyronine (T_3). There was also some indication that there was inherent variability between different meal varieties, but that the development of protein concentrates offered some of the best potential to alleviate glucosinlate levels (Forster et al., 1999).

With Australian canola meals the maximum level of dietary inclusion of either solvent or expeller extracted canola meals was shown to be at least 60% of the diet, though higher levels were not examined (Figure 5; Glencross et al., 2004b). The levels of inclusion of either canola meal reported in this project are considerably higher than that of any other reported study (Higgs et al., 1982; 1983; Burel et al., 2000; 2001). In part this high inclusion level was accommodated by the protein-limiting specifications of the diet in this study, but regardless the overall dietary inclusion levels are significant given that other researchers have had difficulty including more than 40% canola meal in diets for any fish species.

Overall, canola/rapeseed meals do offer some potential for use in fish feeds, proivided they are cost competitive with other raw amterials on a protein and energy basis. The primary negative point with canola meals is a reputed problem with glucosinolate effects on fish thryoidal metabolism. However, differing results have been obtained in Australia, Canada and Europe with the assessment of these meals. Further complicating the assessment is that each of these studies has also used a different fish species.

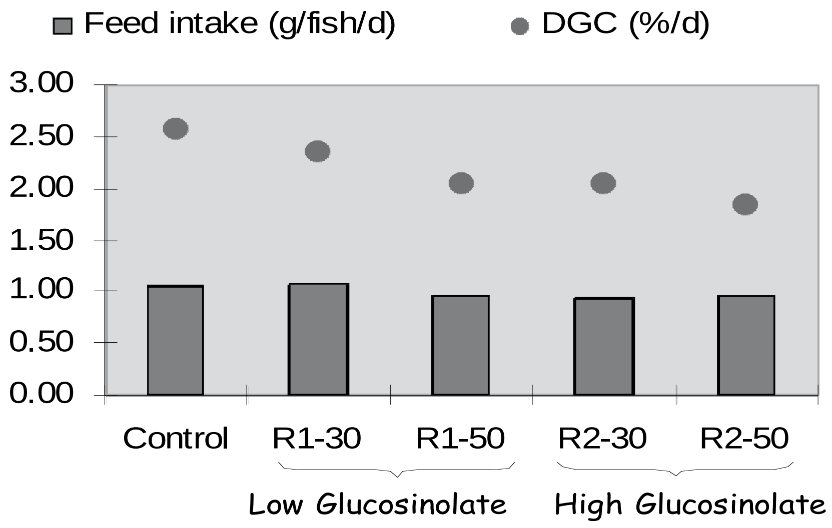

Figure 3. Effect of low and high glucosinolate varieties of European rapeseed meal on the growth rate (DGC) and feed intake by trout.

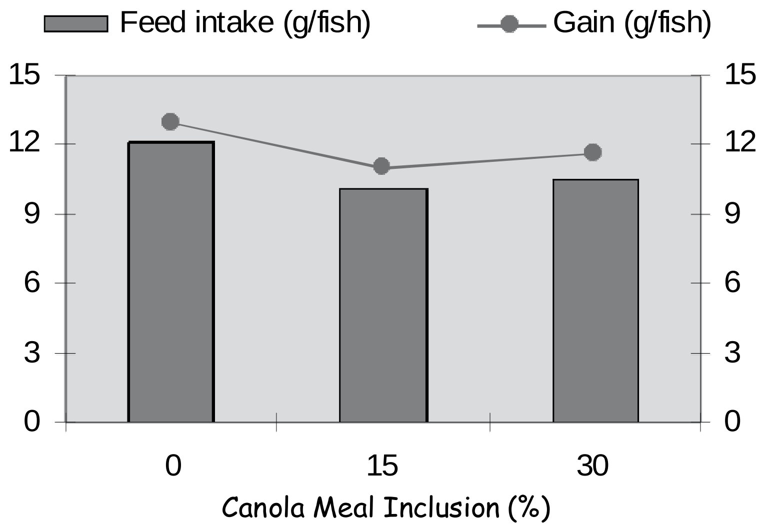

Figure 4. Effect of inclusion of candian canola meal on the weight gain and feed intake by chinhook salmon. From Higgs et al., (1983).

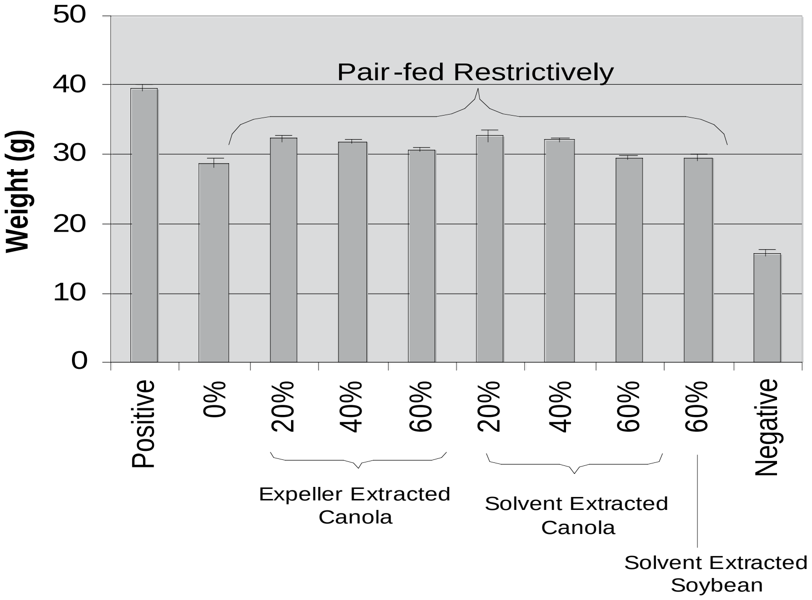

Figure 5. Effects of different canola meal types on the nutritional value of the meal under pair-fed feeding regimes. Notable is that the protein value determined using this approach demonstrates that neither processing method results in any greater reduction in protein value than that of soybean meal.

Anti-Nutritional Factors in Canola Meals

A variety of anti-nutritional factors (ANF) are found in canola meals (Table 2). Canola meals in particular are noted for their glucosinolate content. Glucosinolates in their own right have little biological activity. The actual anti-nutritional components are the breakdown products of glucosinolates, such as isothyocyanates, nitriles, thiocyanate anions and vinyloxazolidinethiones, which all have some goitrogenic activity. Effectively these compounds induce hypo-thyroidism in most vertebrate animals and lead to reduced levels of the thyroid hormones triiodothyronine (T_3) and thyroxine (T_4).

Table 3. Anti-Nutritional Factors (ANF) found in key feed grain varieties.

Anti-Nutrient (mg/kg DM)	Soybean	Lupin kernel	Rapeseed	Field Pea
Alkaloids	40	100	20	10
Glucosinolates	-	-	9,000	-
Lectins (dilutions)	-	-	-	4
Oligosaccharides	62,000	70,000	11,000	35,000
Phytate	9,000	5,000	40,000	5,500
Protease Inhibitors	10,000	2,000	10,000	9,000
Saponins	5,000	570	-	-
Tannins	0	0	1,500	8,000

Changes in the levels of plasma thyroid hormone, triiodothyronine (T_3) and thyroxine (T_4) have been observed from fish fed incremented levels of rapeseed meal (Burel et al., 2001). In addition to the changes in thyroid hormone levels, poorer growth and feed utilisation was also observed in fish from the rapeseed meal fed treatments.

The addition of the enzyme phytase to the diet of fish can result in significant improvements in the digestibility of phytate, and at the highest inclusion level of phytase, improvements in phosphorus digestibility are noted (Forster et al., 1999). However, no improvements in digestibility of protein or energy are noted. The effect of phytate as an anti-nutritional factor in fish diets still remains and needs further consideration.

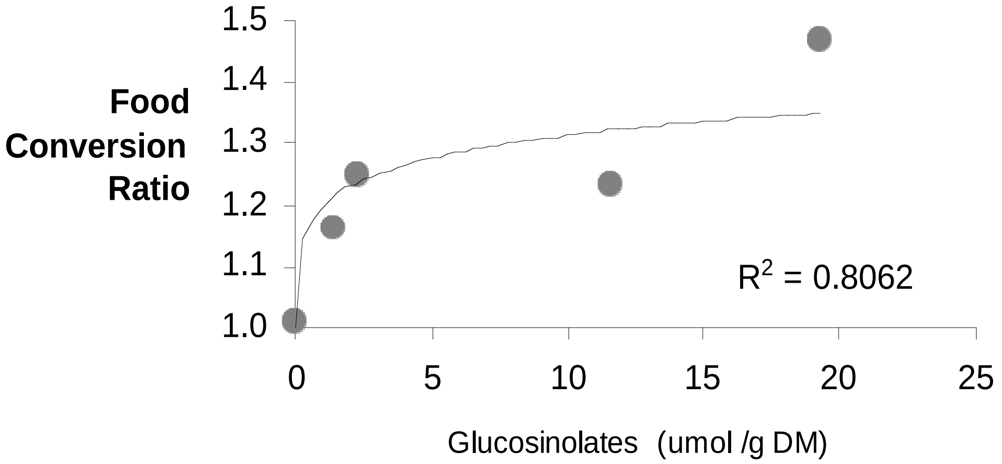

Figure 6. Effect of glucosinolates on food conversion ratio of diets fed to rainbow trout.

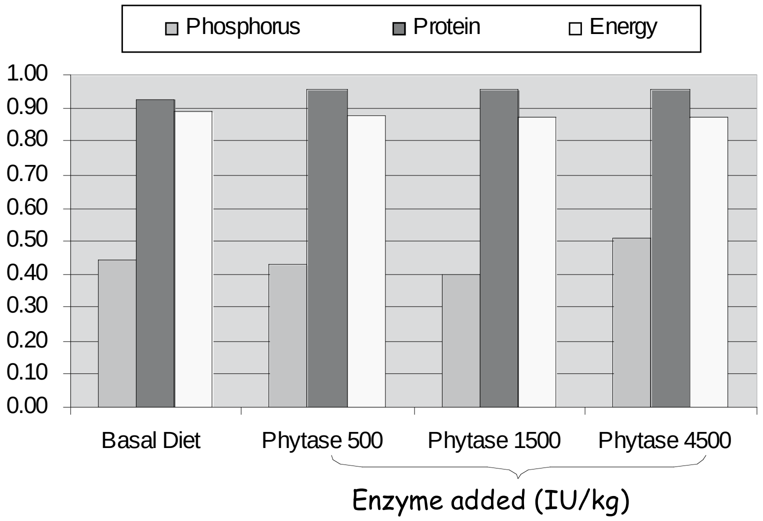

Figure 7. Influence of the addition of the enzyme phytase to diets containing a canola protein concentrate. Notable is the improvement in the digestibility of phosphorus but no changes in the digestibility of protein or energy.

Application of Canola oils to Aquaculture Feeds

Although the replacement of the fishmeal component in aquaculture diets has received considerable research attention in recent years, alternatives to the use of fish oils in aquaculture diets have been comparatively neglected. In some respects, with the present trend towards using high-nutrient-dense diets in aquaculture, there is a more urgent imperative to identifying alternatives to the use of fish oils than there is for fishmeals. A substantial amount of work has focussed on this issue both in Australia and Internationally in recent years (Bell et al., 2003; Glencross et al., 2003; Grisdale-Helland et al., 2002).

The inclusion of plant oils, such as canola or soybean oil in aquaculture diets is not a new concept, however consolidation of information on this issue and widespread industry adoption remain a high priority for the aquaculture industry (Naylor et al., 2000). However the replacement of fish oil in diets of fish, particularly marine fish, is likely to present further complications. Most fish have shown an essential requirement for some of the polyunsaturated fatty acids (PUFA) as they lack the enzymes required to make these PUFA at the required rate to sustain fast growth (Sargent et al., 1999).

Canola oils are one oil resource with considerable potential for use as replacers of fish oil in diets for fish. Similar to soybean oils, canola oils are also deficient in any of the long-chain PUFA (lcPUFA) such as EPA (eicosapentaenoic acid; 20:5n-3) and DHA (docosahexaenoic acid; 22:6n-3). While canola oils have appreciable quantities of the short-chain PUFA of both linoleic acid (LOA; 18:2n-6) and linolenic acid (LNA; 18:3n-3), the proportions that they represent within the oil are substantially different to that of soybean oils, with canola oils containing a greater content of LNA.

In most of the studies published to date, the substitution of fish oil by grain-derived oils does not impair the growth of fish fed these diets (Bell et al., 2003; Glencross et al., 2003a). Most of the international work in the area has focussed on rapeseed/canola oils, with some further work on other oil resources like soy and palm. The requirement for essential fatty acids in grow-out diets for most fish appears to be satisfied by the basal inclusion level of some fishmeal in the diets, which contributes a small, but sufficient amount of the required EPA and DHA needed (Sargent et al., 1999).

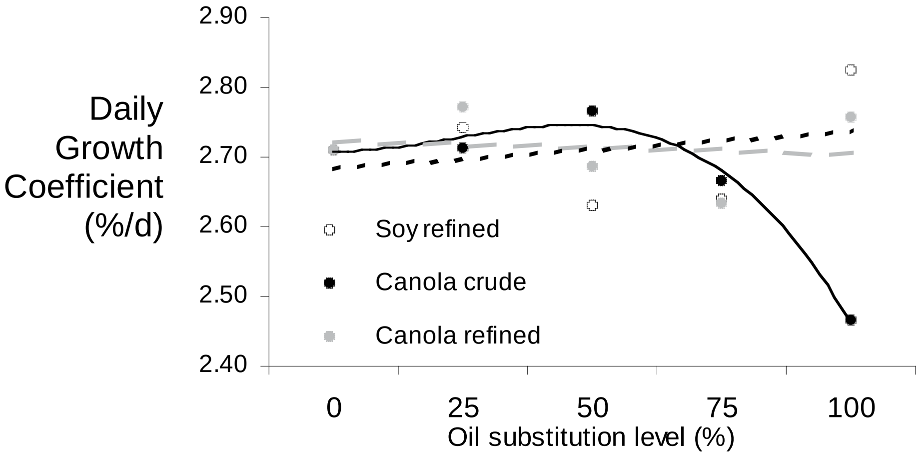

Figure 8. Effect of dietary fish oil replacement by crude and refined canola oil, and soybean oil on growth of Pink snapper.

With most dietary lipids, the fatty acid composition of the animal becomes that of what it eats. This has certainly been the case also with fish oil replacement studies; where the use of canola and soybean oils has changed the fatty acid profile of the fish (Bell et al., 2003; Glencross et al., 2003a).

In studies examining the effect of fish oil replacement by canola or soybean oils on the flesh composition and flesh sensory characteristics of Pink snapper, it was shown that both oils had a significant effect on both parameters (Glencross et al., 2003a). Notable changes in sensory parameters of flesh colour, flavour oiliness, flavour strength, texture moistness and texture flakiness were all observed (Figure 9). Ironically an overall assessment of acceptability had both canola and soybean oil fed fish being more popular than the fish oil fed fish (Glencross et al., 2003a).

To ameliorate this problem the use of “finishing” diets has been touted and the evidence accumulated does suggest that this strategy has some potential (Bell et al., 2003; Glencross et al., 2003b).

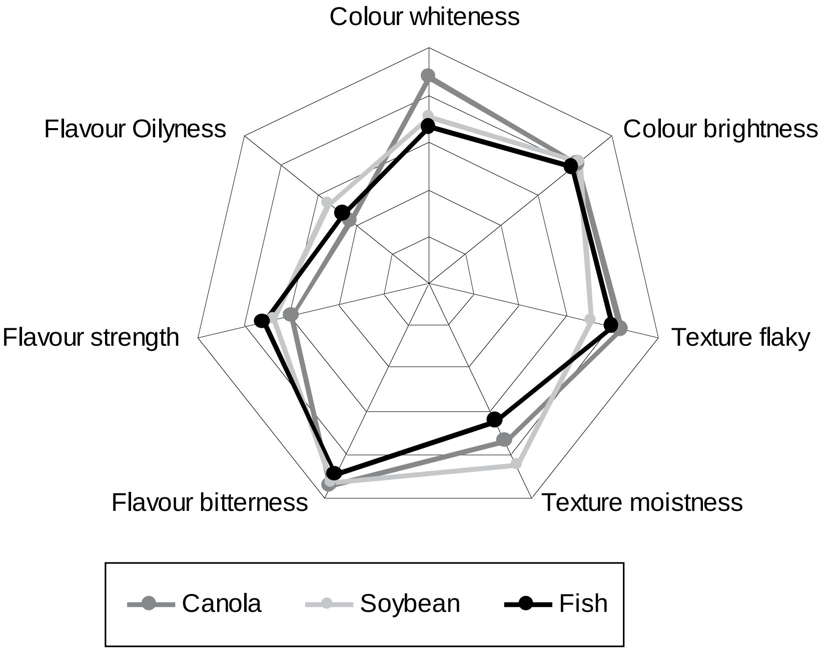

Figure 9. Effect of dietary fish oil replacement by refined soybean or canola oil on the sensory qualities of Pink snapper fillets.

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Shrimp and Feed Grains

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Introduction

The need for viable alternatives to fishmeal in aquaculture feeds for shrimp was highlighted during the *El Nino* years when world fishmeal production dropped from 6.8 million tonnes in 1996/97 to 5.1-5.2 million tonnes in 1997/98 (FIN, 2006). More recently the huge increase in demand for fishmeal from China has resulted in the price of premium grades of fishmeal that are used in aquaculture feeds increasing from about US$720/ton FOB Peru in December 2005, to the current price of about US$1150/ton FOB Peru (Hammersmith Marketing, 2007). The cost of other marine protein sources has also risen apparently because of increased demand. Typically plant protein meals are minor components in shrimp feeds. Even soybean meal, the most commonly used plant protein, until recently, generally constituted less than 10% of the feed. In favourable rainfall years, Australia produces substantial quantities of lupins (1.3 mt), field peas (0.4 mt) and canola (1.7 mt), and relatively small amounts of soybean. It would be of great benefit to the Australian grain legume industry if these protein-rich grains could be used cost-effectively to replace a significant proportion of the fishmeal in prawn feeds.

Issues relating to shrimp feeds

The most widely grown species of shrimp in south-east Asia are Pacific white shrimp, *Litopenaeus vannamei* and black tiger shrimp, *Penaeus monodon,* while in Australia *P. monodon* is the dominant species, with some farmers growing the banana shrimp, *P. merguiensis*. The feeds for these shrimp are characterised by relatively high protein content (32% to 38% on an ‘as used’ basis, relatively low lipid content (6% to 8%) and they generally contain a significant amount of starch and fibre. Generally, about half the protein in the feed is provided by the inclusion of fishmeal in the formulation. The balance is made up from other marine animal products (crustacean meals or squid meal) and plant protein sources (soybean meal, limited amount of gluten), and endogenous protein in wheat and other minor ingredients. Hence, there is great potential to replace imported fishmeal and soybean meal in the shrimp feed formulations with alternative, Australian-produced ingredients that are rich in protein – ingredients such as lupin kernel meal, field peas and possibly canola meal.

Feeds for Atlantic salmon contain high levels of lipid and protein and thus there is little space in the formulation for “fillers” of low nutritional value, such as starch and fibre. Hence, formulators seek ingredients with relatively high protein or fat content. This is in contrast to the situation with shrimp feeds, where ingredients containing as little as 36% crude protein can be included in the formulation. These ingredients bring with them significant amounts of starch and fibre. The starch has a role in the shrimp feeds as it is used to a moderate extent by the shrimp as an energy source and it acts to hold the feed pellet together through its binding action. However, careful consideration needs to be given to the use of ingredients with high fat content, in order that the dietary lipid content of the feed is maintained below 8% and the fatty acid composition of the lipid remains within the limits considered optimal.

A key issue in the selection of a protein-rich ingredient in any aquafeed formulation is its cost in terms of cost per unit weight of protein. The current cost of soybean meal that is imported from the USA is about A$445/t, and hence the cost of the protein is about A$810/t. This is much lower than the cost of protein from high quality Peruvian fishmeal which is about A$2230/t (67% protein, A$1560/t for fishmeal). Hence, even a moderate inclusion of these plant ingredients would result in a substantial reduction in the ingredient cost of shrimp feeds. The Australian aquaculture industry produces about 3500 mt of shrimp each year, using about 6000 mt of feed (ABARE, 2006). This feed is either manufactured locally (Ridley Aquafeeds) or imported from south-east Asia. Clearly, this Australian market is very small in the global or regional sense, with Asia currently producing about 1.66 m mt of shrimp, using about 2.4 m mt of feed. As the cost of fishmeal has almost doubled in the last 2 years, there appears to be an opportunity for Australian-produced feed grains to be used to a greater extent by both local and by south-east Asian aquafeed manufacturers.

Grains that have been evaluated with shrimp

Plant protein meals differ nutritionally from fishmeal, most noticeably in having a less favourable amino acid profile, having a high content of relatively indigestible carbohydrate, containing none of the nutritionally-important, long chain highly unsaturated fatty acids, and containing some anti-nutritional factors. Furthermore, diets containing high proportions of plant proteins are not well accepted by shrimp (Smith et al., 2005) unless they contain one or more ingredients that contain feeding stimulants or chemoattractants that will elicit a strong feeding response from the shrimp. However, there is an increasing body of research into the use of plant protein sources as partial replacements for fishmeal in aquaculture feeds for shrimp. The earliest studies were with soybean meal (Lim and Dominy, 1990; Chamberlain, 1995; Paripatananont et al., 2001; Samocha et al., 2004). More recently there have been studies with field or feed peas (*Pisum sativum*) (Cruz-Suarez et al, 2001; Davis et al., 2002; Bautista-Teurel et al., 2003), canola (Buchanan et al., 1997; Lim et al., 1997; Cruz-Suarez, 2001) and lupins (Sudaryono et al., 1999a,b,c; Smith and Tabrett, 2003). A broad study into the use of alternative protein sources in shrimp feeds that included canola, soybean, field peas, canola and lupins was carried out in Australia as part of an FRDC research program into the replacement of fishmeal in aquaculture feeds (Smith, 1998; Smith et al., 2001). A further study with support from the GRDC was carried out specifically to investigate way to improve the efficacy of lupins as fishmeal replacements in shrimp feeds (Smith, 2002).

Digestibility of plant protein sources

The digestibilities of a number of plant protein sources when included in shrimp feeds were determined by Smith et al. (1998). These showed that the protein in both lupin kernel meal and solvent-extracted soybean meal were high (>92%), whereas that of canola meal was significantly lower (Table 1). The digestibility of lupin kernel meal was also clearly greater than that of whole seed meal. Recent studies into the new cultivars of *L. angustifolius,* that have replaced the older cultivar Gungurru, have shown that the protein digestibility of the kernel meals were uniformly high with an average of 94% (Smith et al. 2007). The apparent dry matter digestibility and apparent digestibility of energy were less than that of protein (62% and 74%, respectively) due to the amount of non-starch polysaccharides present in the kernel meals.

Various processing methods have been applied to feed peas to determine the extent that the digestibility of the peas could be improved through processing (Cruz-Saurez et al., 2001; Davis et al. 2002; Bautista-Teruel et al., 2003). These studies, with three different species of shrimp, showed that dehulling the peas significantly improved digestibility, but additional improvements could be gained through extruding and infrared cooking of the peas. The apparent protein and energy digestibility of the peas that were dehulled, extruded and cooked was similar to that reported for lupin kernel meals (Cruz-Saurez et al., 2001). Cruz-Suarez et al. (2001) also determined the apparent crude protein digestibility of extruded canola meal and found it to be lower than that of the dehulled, extruded field peas (79% cf. 92%). This was consistent with the findings with canola reported by Smith et al. (1998).

Table 1. Apparent digestibilty (%) of dry matter, crude protein, energy and lysine in protein-rich feed ingredients of plant origin. Estimates are from diets prepared using modified preparation procedure. Results are from chromic oxide data. Pooled standard errors of the means are in parentheses. Lupins are *L. angustifolius* cv. Gungurru.

Ingredient	Dry matter	Crude protein	Energy	Lysine
Lupin (whole)	39 (1.7)	88 (0.6)	45 (1.4)	87
Lupin (dehulled)	73 (1.7)	95 (0.6)	74 (1.4)	95
Soybean (defat.)	64 (1.7)	92 (0.6)	72 (1.4)	94
Canola	42 (1.8)	78 (0.6)	49 (0.9)	56
Field pea (Dunn)	72 (1.8)	89 (0.6)	83 (0.9)	83
Gluten (wheat)	96 (1.8)	102 (0.6)	100 (0.9)	100

Nutritional value of plant protein sources

Smith (2002) found that the growth rate of shrimp fed diets in which fishmeal had been replaced with lupin kernel meal (*L. angustifolius* cv. Gungurru) decreased progressively with inclusion level and recommended a maximum inclusion level of about 25% of the diet. Recently the nutritional value of twelve samples of lupin kernel meals, including seven new cultivars of *L. angustifolius* currently being grown in Western Australia and a sample of *L. luteus* cv. Wodjil were evaluated in feeds for black tiger shrimp *P. monodon*. Two growth response experiments were carried out to compare a fishmeal-based basal diet with diets in which the lupin kernel meals replaced over half the fishmeal in the basal diet on a protein-equivalent basis. These kernel meals were included in the feeds at high levels (~40% of the diet), and they performed just as well as the basal diet. These results demonstrated that the compound or compounds present in the earlier cultivar Gungurru that caused the decrease in performance of the shrimp, were not present in the new cultivars. In a third experiment, the performance of diets containing a kernel meal prepared from a 'typical' commercial mixture of lupins (Cooperative Bulk Handling, Forrestfield, WA), and one of the new cultivars (Kalya) were compared with that of solvent extracted soybean meal at two protein-equivalent inclusion levels. The feeds contained the same amount of protein, but with ~24% and ~42% of the dietary protein provided by soybean meal and by the lupin kernel meals. There were no significant differences in the growth rates among the treatments containing soybean meal or lupin kernel meal, irrespective of the inclusion level. The replacement of fishmeal in the basal diet with soybean meal and with lupin kernel meal did not have an adverse effect on performance. In fact, with the diet containing the lower inclusion level of the cultivar Kalya (25% of diet), the growth rate was significantly greater than for the basal diet (Figure 1). The results demonstrate that solvent–extracted soybean meal and lupin kernel meal from the new cultivars of *L. angustifolius* could be used interchangeably in feed formulations, and reinforced the findings that these plant protein meals could be used to provide at least 40% of the dietary protein in shrimp feeds.

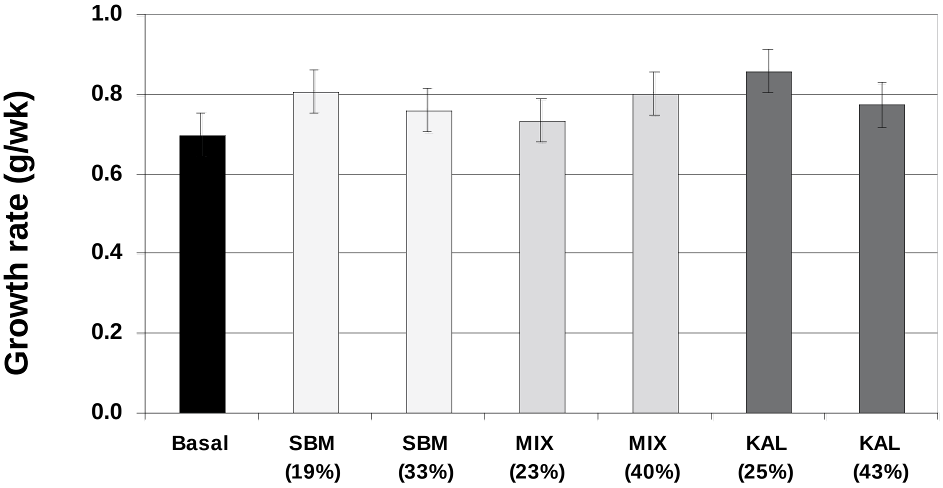

Figure 1. Growth rate of shrimp (*Penaeus monodon*) fed diets in which fishmeal protein in the basal diet had been replaced on a protein-equivalent basis with solvent-extracted soybean meal (SBM) , and two samples of lupin kernel meal (*L. angustifolius*) (MIX = Mixed cultivars, KAL = cultivar Kalya). The inclusion level of the plant protein sources is shown in parenthesises.

Bautista-Teruel et al. (2003) carried out a dose-response study with post-larval black tiger shrimp, in which they serially replaced the 25% inclusion of defatted soybean meal with feed pea meal. The replacement was carried out on a protein-equivalent basis. The study showed no significant effect on growth or survival even with total replacement of the soybean meal. Sudaryono et al. (1999c) carried out a similar study replacing a 30% inclusion of defatted soybean meal with the kernel meal from white lupins *L. albus.* Their data show a progressive decrease in growth rate of *P. monodon* juveniles with increasing replacement of soybean meal. High-fibre and low-fibre canola meals were evaluated in a study with the Pacific white shrimp, *L. vannamei* (Lim et al., 1997). This study showed that there was a progressive decrease in shrimp growth and feed intake with increasing replacement of dietary fishmeal with canola meal on a protein-equivalent basis. The authors recommended that dietary inclusion of the high-fibre canola meal be limited to provide no more than 15% of the dietary protein or about a 14% inclusion in the feed. However, the authors postulated that "…*one or more fibre-reduced, and solvent-extracted canola protein products may be cost-effective substitutes for fishmeal protein*" (Lim et al., 1997). All of the above studies were carried out with diets that contained some marine animal protein that was held at a constant inclusion level within the series of diets used in an experiment. It appears that there is a need for some marine animal product in shrimp feeds to provide attractants and some unidentified components that provide nutritional benefit (Smith et al., 2005; Williams et al., 2005). Hence it is unlikely that the plant protein sources will be able to be used to fully replace all marine animal protein sources in shrimp feed formulations.

Summary

In conclusion, this review has revealed a great consistency in the results of experiments assessing the nutritional value of similar plant protein meals across three species of shrimp. This means that there is a high likelihood that results obtained with one species using properly designed and run experiments, can be applied to another shrimp species. Furthermore, it has demonstrated the usefulness of solvent-extracted soybean meal, lupin kernel meal and heat-processed dehulled field peas to partially replace fishmeal in shrimp feeds. These ingredients appear to be interchangeable on a protein-equivalent basis. It does not appear likely that there will be a total replacement of marine animal protein sources (fishmeal, squid meal or crustacean meal) from shrimp feed formulations, rather a significant replacement of fishmeal with plant protein sources complemented with some other marine products. However, there will be need for further research to develop a product from canola meal that can be used with confidence to replace fishmeal protein. Finally, the factors that will affect the use of solvent-extracted soybean meal, lupin kernel meal and defatted and dehulled feed pea meal in shrimp feeds will be the cost of the protein ($/kg) and the amount of "filler" that can be accommodated in the formulations.

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Grains and summer temperatures in Atlantic salmon

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Introduction

Due to climate change and increased summer water temperature understanding how this affects the nutrition of Atlantic salmon is a major issue world wide and impacts on farming in all major producing countries including Norway, Scotland and Chile. It is a particular concern in Tasmania where salmon are farmed at the extreme of their natural temperature range. Further to this, the need to replace fish meal and development of plant proteins has posed many fundamental and practical questions about fish nutrition and aquafeeds. A “normal” summer temperature of 15°C reflects standard conditions for Tasmania, it is higher than has usually been considered in other parts of the world and is a temperature we have used as a reference for previous research (e.g. Carter and Hauler 2000; Glencross et al. 2005). This is in contrast to a “high” summer temperature of 18-19°C at which Atlantic salmon will still feed and grow but can be considered at the extreme of the temperature range where this is done commercially in Tasmania. One area that requires investigation concerns the combined effects of ingredients and temperature. Consequently we have investigated the utilisation and biological value of lupins at normal and high summer temperatures for Atlantic salmon. The lupin research is a component of our research aimed at gaining a greater understanding of many aspects of temperature and fish nutrition (e.g. Carter et al. 2005; Miller et al. 2006).

Experiments

Research on the use of lupins by Atlantic salmon at summer temperatures was designed to provide a comparison between selected lupin species, varieties and processing methods as well as with dehulled soybean meal, as a competing and well established plant protein. The experiments described briefly below measured apparent digestibility; tested whether lupins affected the rate of gastrointestinal evacuation; and measured salmon growth performance and the biological value of lupin products at normal and high summer temperature.

Apparent Digestibility

Ingredients tested. Experiment AD05: *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). Experiment AD06: *L. angustifolius* (cv. Gungarru) kernel meal (GKM), *L. angustifolius* (cv. Mandelup) kernel meal (MaKM), *L. angustifolius* (cv. Myallie) kernel meal (MKM), *L. angustifolius* (cv. Tanjil) kernel meal (TKM), and *L. angustifolius* (cv. 2173M) kernel meal (2173KM).

Apparent digestibility (AD) experiments followed a standard approach for measuring AD of ingredients in aquafeeds (Austreng et al. 1978; Percival et al. 2001). In summary, seawater salmon were held at 15°C and fed either a reference feed or an experimental feed made from the reference feed and 30% of the ingredient to be tested. After 9 days on the experimental feed salmon were “stripped” of faeces, chemical composition of the faecal samples analysed and AD values calculated, AD values for crude protein showed significant differences between ingredients (Table 1). In AD05 L. luteus kernel meal and protein concentrate were better digested than a *L. angustifolius* kernel meal (MKM) and a soybean meal. Experimental design was improved for AD06 and provided greater resolution of differences between ingredients and data indicated 3 *L. angustifolius* meals had superior AD values for crude protein with GKM (cv. Gungarru) being the highest.

Table 1. Apparent digestibility (AD, %) for lupin and soybean fed to seawater salmon during two experiments, AD05 and AD06.

AD05	AD Crude protein	**AD06**	AD Crude protein
LPC	85.96[a]	GKM	94.76[a]
	1.18		2.96
LKM	83.09[a]	MaKM	66.06[c]
	0.53		3.11
BKM	76.97[ab]	MKM	80.58[b]
	1.17		5.21
MKM	72.41[b]	TKM	80.83[b]
	0.41		2.32
SBM	70.15[b]	2173KM	67.89[bc]
	3.87		2.72
P^a	<0.01	P^b	<0.001

P^a, Mean ± SEM (n=2). P^b, Mean ± SEM (n=3).

Experiment AD05: *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). Experiment AD06: *L. angustifolius* (cv. Gungarru) kernel meal (GKM), *L. angustifolius* (cv. Mandelup) kernel meal (MaKM), *L. angustifolius* (cv. Myallie) kernel meal (MKM), *L. angustifolius* (cv. Tanjil) kernel meal (TKM), and *L. angustifolius* (cv. 2173M) kernel meal (2173KM).

Gastrointestinal Evacuation Rate

Atlantic salmon pre-smolt were obtained from Wayatinah Salmon Hatchery (SALTAS, Tasmania, Australia). One hundred and forty four fish were distributed between 12 300-l conical bottomed tanks at 12 fish per tank (144.2 ± 5.8 g). The fish were acclimated to seawater over 4 days then hand fed a commercial salmon feed for 6 weeks. Fish were held at the School of Aquaculture in a partial recirculation system and water treated with physical, biological and UV filtration. Water temperature was controlled at 15.0 ± 1.5 °C and fish were exposed to controlled photoperiod, L:D 16:8h. Water quality (dissolved oxygen, pH, salinity, ammonia, nitrite, nitrate) was monitored regularly and maintained within recommended limits for the experiments.

A reference mash was formulated and 5 experimental diets made to include 30% of each test ingredient. Two sets of reference mash containing either 0.1% Yttrium oxide or 0.1% Ytterbium oxide as inert markers were used to make extruded feeds. Ingredients tested were those tested in AD05: *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). For logistical reasons the two sets of feeds with different markers were made on different extruders. To ensure similarity in pellet dimensions the feeds were re-pelleted to 4.5 mm diameter using a California laboratory pellet mill (CL-2), dried at 35°C for 16 h and stored at below 4°C.

The GER experiment was based on the replacement of one marker with a second marker in the same feed, faecal collection over time after the change in marker and the measurement of the 2 markers in faecal samples (Storebakken et al. 1999). Fish were held in 350-L conical bottomed tanks fitted with Guelph type faecal collectors (Carter and Hauler 2000). Ytterbium-labelled (Marker 1) feed was fed via belt-feeders twice per day for 8 days, on day 9 the feed was replaced by the Yttrium-labelled (Marker 2) feed and faecal samples taken over the following 28 h at 0, 4, 8, 12, 16, 20, 24 and 28h. GER was expressed as the percent of the sum of the two markers that was accounted for by the second marker (M2). Regression analysis was conducted using Sigmaplot according to the model: $M2\ (\%) = (Max - Min) / ((1 + (T/T_{0.5})^{-b}) + Min)$ where M2 is the percent of marker 2, Max and Min are the upper and lower asymptotes, T the time in hours and $T_{0.5}$ the time at which half the marker was M2, and b the slope (Figure 1).

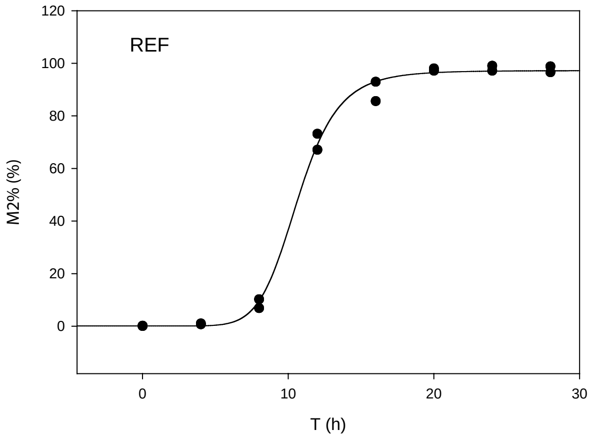

Figure 1. Evacuation curve for REF (M2% - marker 2).

The model successfully described the evacuation of diets containing 30% of the different ingredients (Table 2). There were differences between ingredients. The plateau (Max) showed how the meals had been flushed out of the gastrointestinal tract and lower values suggested more mixing had taken place between discrete meals. The plateau (Max) was lowest for soybean and highest for LKM. The three kernel meals (KM) took less time for 50% to be evacuated ($T_{0.5}$) when compared to the fish meal, soybean meal and the lupin protein concentrate. The slope also differed between ingredients but not as distinctly as for $T_{0.5}$, the kernel meals had the lowest slopes where as the lupin protein concentrate was higher than the other ingredients. Higher slope values indicated a faster more uniform movement of a single meal where as the $T_{0.5}$ value was more indicative of when the process started.

Table 2. Best fit of parameters for gastrointestinal evacuation rates (percent marker) for Atlantic salmon using asymptotic regression curve.

Diet	Min (%)	Max (%)	$T_{0.5}$ (h)	Slope (-ve)	n	R^2	P
REF	0.1	97.09	10.68	7.67	16	99.5	<0.001
SBM	0.4	88.52	11.43	8.59	15	95.8	<0.001
LPC	0.4	92.60	10.17	10.72	15	98.0	<0.001
LKM	0.1	97.80	8.55	6.48	16	99.8	<0.001
BKM	0.4	91.92	8.82	6.54	16	94.0	<0.001
MKM	0.2	93.53	8.48	5.60	15	96.9	<0.001

Model: Marker 2 (% total marker) = (Max – Min) / ((1 + $(T/T_{0.5})^{-b}$) + Min). (M2: Y_2O_3) as a percent of total faecal marker for fish meal reference (REF), *L. luteus* (cv Wodjil) kernel meal (LKM), *L. luteus* protein concentrate (LPC), *L. angustifolius* (cv. Belara) kernel meal (BKM), *L. angustifolius* (cv. Myallie) kernel meal (MKM) and soybean meal (SBM).

Interestingly the slope calculated for the REF diet in the present study was similar to that for a fishmeal diet fed to Norwegian Atlantic salmon (Storebakken et al. 1999): 7.5 and 7.7 h respectively. However, $T_{0.5}$ was nearly 8 hours less in the present study, 10.7 h compared with 18.2 h. Similarly, for soybean the slopes were 8.6 and 11.0 h, respectively, and $T_{0.5}$ 11.4 and 19.8 h, respectively (Storebakken et al. 1999). The longer $T_{0.5}$ was probably explained by the lower temperature of 9°C for the Norwegian salmon, digestion and GER were slower at the lower temperature. It is note worthy that the slope appeared to reflect the ingredient regardless of temperature, soybean had a higher slope than fishmeal. However, this is a preliminary observation and there are no other data available in the literature to expand further. Interestingly, there was a relationship between crude protein AD of the diet and the diets' slope values that suggested diets (ingredients) which were more digested were moved through the gastrointestinal tract more quickly once this process began (shown by $T_{0.5}$). Again, this preliminary observation requires further testing. Overall the results indicated that ingredients influenced both the time for 50% of a feed to be evacuated and the speed at which evacuation occurs once the process has begun.

Biological Value

The experiment aimed to compare the biological value of a lupin kernel meal (*L. angustifolius* cv Coromup) with fishmeal and with soybean at two temperatures and two inclusion levels. Inclusion levels of lupin and soybean were 15 and 25% at 14°C and 15% at 18°C. Diets were formulated to be isonitrogenous and isoenergetic on a gross compositional basis and to have a marginal crude protein content (40%). Inclusion of 15% reflected maximum industry inclusion rates for lupin where as 25% inclusion reflected a higher level in order to investigate whether performance changed at the higher level. The lower temperature of 14°C reflected a normal summer temperature and was compared with a high summer temperature of 18°C at which salmon would still be fed.

Table 3. Preliminary analysis of Atlantic salmon performance when fed lupin or soybean at different temperatures and inclusion levels (weeks 4 to 8).

	14°C					18°C			
	FM	**COR 15**	**COR 25**	**SB 15**	**SB 25**	**FM**	**COR 15**	**SB 15**	**P**
Initial Weight (g)	197.40	196.90	193.50	202.80	197.30	205.90	198.90	201.10	ns
	2.30	4.50	5.90	4.20	4.70	6.60	4.00	4.90	
Feed intake (%BW/d)	0.88^{a}	1.32^{bcd}	1.44^{cd}	0.98^{ab}	1.04^{ab}	1.07^{abc}	1.47^{d}	1.12^{abc}	<0.001
	0.08	0.05	0.19	0.06	0.04	0.02	0.01	0.03	
Growth rate (%BW/d)	0.71	1.07	1.02	0.66	0.69	0.63	1.00	0.64	<0.050
	0.11	0.09	0.07	0.08	0.11	0.21	0.01	0.03	
FER (g/g)	0.79	0.80	0.72	0.68	0.65	0.57	0.68	0.56	ns
	0.06	0.04	0.04	0.12	0.09	0.19	0.01	0.01	

Mean ± SEM (n=3), multiple comparison by Tukey HSD (significant difference in growth rate not resolved). FM, fishmeal reference; COR, lupin kernel meal (*L. angustifolius* cv Coromup); SB, soybean meal.

Preliminary results for the period 4 to 8 weeks showed that diet had a significant effect on growth performance through differences in feed intake, no significant differences in feed efficiency ratio over this period of the experiment (Table 3).

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Cereal Grain – Functionality and Nutritional Value

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Introduction

The effects of genotype and environment result in substantial variation in the nutritional and functional properties of cereal grain. In this paper, I will refer to research carried out by the Grain Products Laboratory at the Department of Agriculture and Food Western Australia to identify and utilise this variation to advantage.

Proximate composition of cereal grain

The proximate composition of the main cereal grains, wheat, barley and oats, together with oat groats and the minor cereal, triticale, are presented in Table 1. The data are the means of 11 trials grown in 'balanced' trials in Western Australia in 1982 and 1983. The term 'balanced' means that the trials were grown at the same location, on a similar soil type, and had the same sowing date.

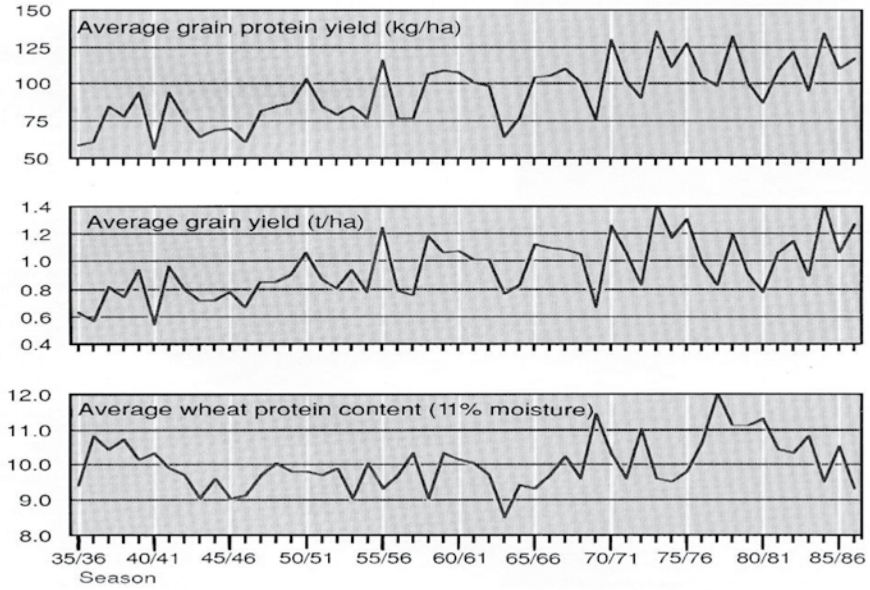
Table 1. Proximate composition of cereal grain – mean data, 11 "balanced' trials, 1982-1983.

Cereal grain	Crude protein	Oil	'Nitrogen-free extractives'	Crude fibre	Ash
Wheat					
cv Gamenya	13.4	1.9	80.0	3.3	1.9
cv Canna	13.1	2.0	79.8	3.1	1.8
Barley					
cv Stirling	11.0	2.7	78.8	5.3	2.2
cv Forrest	11.5	2.3	78.7	5.0	2.5
Oats					
cv West	11.3	5.7	67.5	12.8	2.6
cv Mortlock	11.8	5.7	67.1	12.4	2.6
Oat groats					
cv West	15.0	8.5	72.5	2.0	2.0
cv Mortlock	15.4	8.4	72.4	1.8	1.9
Triticale					
cv Tyalla	13.7	2.4	78.5	3.5	2.1
cv Coorong	12.9	2.1	79.6	3.6	2.0

All values are % on a dry basis

Variation in wheat protein content

The effect of season on wheat protein (expressed as N x 5.7 on an 11% moisture basis) is illustrated in Figure 1, in which average protein levels for the State over the period 1935/36 to 1985/86 are shown – these generally ranged from 9.0 to 11.0%. Over this period, highest levels were achieved in the drought years of 1969, 1972 and 1977. The inverse relationship between yield and protein level is apparent, with an exception being in 1963/64 when a severe stem rust epidemic devastated the crop in many areas. In some areas, those that produced much of the harvested grain, crop yields were high and protein levels were low. However, over the total area sown, the average yield was low – this gave a combination of low average protein and a low average yield for the State.



Source: Crosbie and Fisher (1987).

Figure 1. Average protein content, average yield, and average protein yield for wheat in Western Australia from 1935/36 to 1986/87.

Within paddock variation in protein content from 7.1% to 17.6% was recorded by Parish (1963) at Merredin Research Station in 1962, which he associated with different degrees of moisture stress on the various soil types.

Within the State, highest protein levels are generally achieved in northern and north-eastern areas which have a shorter growing season and lower rainfall; also the lower rainfall more fertile areas in the eastern wheatbelt. Lower protein levels are found in southern and western areas, associated with a longer growing season and higher rainfall.

Variation in wheat protein quality

Whereas wheat protein content is largely influenced by the environment, the composition and functional properties of the protein are determined by the genotype.

When hydrated, wheat protein forms gluten, and the characteristics of the gluten and its interaction with other chemical components including lipids that determines the properties of the dough.

Dough properties are often measured with dough mixing equipment (Farinograph or Mixograph) or dough stretching equipment (Extensograph) or can be predicted by various small-scale tests.

High protein content and protein quality, combined with moderately high damaged starch levels are required to achieve high quality conventional bread – here the gluten forms the framework of the loaf of bread and, if of suitable quality, ensures good gas retention and loaf volume.

Application of wheat as a binder in fish feed

Wheat of high protein content and dough strength is often the choice when used as a binder in shrimp feeds. Some 30 years ago, I reduced the effect of rain damage on snail pellets by changing the wheat used as a binder in the formulation to a high protein, strong gluten type. Those considering the use of wheat in fish feeds need to be aware of the considerable variation in wheat types available that could influence pellet cohesiveness.

Wheat starch

Wheat starch, the major chemical component of the wheat grain, was once thought to be relatively unimportant. It is now known to have a key role in influencing the suitability of flour for use in wide range of products.

Starch has two forms: a linear form – amylose; and a branched form - amylopectin. Factors influencing starch functional properties include:

- the ratio of amylose:amylopectin;
- the extent to which amylose is complexed with phospholipid;
- the degree of branching in amylopectin.

Important functional properties include pasting and swelling. Pasting properties may be measured on the Rapid Visco Analyser (RVA). The Flour Swelling Volume (FSV) test provides a rapid means of characterising wheatmeal, flour or starch in relation to the extent of starch swelling that occurs when the sample is heated in the presence of a sufficient volume of water.

The ratio of amylose:amylopectin in wheat starch is associated with three genes, which control formation of amylose. Waxy wheat, in which none of the genes are active (triple-null types) and contain 100% amylopectin, were first developed in 1994. Western Australia, which has long had the reputation as the world's leading supplier of wheat for Japanese udon, has developed this reputation through its breeding of high-FSV wheat varieties, with a slightly increased amylopectin level. Such varieties, in combination with the right protein level and protein quality, produce the desired textural properties in udon.

From a nutrition point of view, attention is being given to the development of wheat varieties containing a higher than normal proportion of amylose. Such types are associated with increased resistant starch levels and lower glycemic index. If animal nutritionists are keen to regulate the breakdown of starch, they need to be aware of the current range in starch quality among wheat varieties. Another factor here for consideration, if using ground wheat or flour, is the level of damaged starch. Higher levels of damaged starch occur in the milling of hard wheats compared with soft wheats. In the formation of pellets by extrusion, starch may be gelatinised if in contact with sufficient water – here starch gel formation may contribute to pellet cohesion, and genetic variation in the characteristics of the gel may be of significance.

Utilisation of oats in animal feeding

Oats have a relatively high crude fibre content, associated with the high proportion of hull in the grain (about 25 to 30% of the weight). In the early 1980s, the hull was considered to have low digestibility, due to its relatively high lignin and silica content. The work of Van Soest in the 1970s, involving the recognition of new measures of fibre and its components: – neutral-detergent-fibre (NDF), acid-detergent-fibre (ADF), and acid detergent lignin (ADL) provided a new insight into the utilisation of fibre by animals. While ruminants are able to digest cellulose, this is limited by the extent to which the cellulose is lignified.

Our inclusion of the Van Soest fibre measurements and an invitro-digestibility test on the 'balanced' trials from 1982 and 1983 provided some interesting results (Table 2).

Table 2. Crude fibre, NDF, ADF, ADL, and in-vitro digestibility levels in cereal grain.

	Crude fibre	Acid detergent fibre	Neutral detergent fibre	Acid detergent lignin	In vitro digestibility
Wheat					
cv Gamenya	3.3	3.9	13.1	1.0	85.6
cv Canna	3.1	3.4	12.3	0.9	86.7
Barley					
cv Stirling	5.3	6.0	17.0	1.2	84.3
cv Forrest	5.0	6.3	17.4	1.3	84.3
Oats					
cv West	12.8	15.2	33.0	1.7	76.9
cv Mortlock	12.4	15.2	32.2	2.3	69.0
Triticale					
cv Tyalla	3.5	4.0	13.1	1.4	88.8
cv Coorong	3.6	3.6	13.2	1.2	85.8

All values are % on a dry basis.

For wheat, barley and triticale, there was little variation between the two cultivars selected in the various fibre measurements. However, there was a marked difference in levels of ADL and in-vitro digestibility between West and Mortlock oats.

Further investigation led to our finding of a 10-fold variation in the level of lignin in oat hulls among Western Australian varieties (Crosbie et al., 1985). Mean levels of ADL ranged from 8 g/kg in Swan to 76 g/kg in Mortlock. This was associated with pepsin cellulase digestibility levels of 293 g/kg and 108 g/kg, respectively. Later studies involving another low lignin variety, Murray, and the high lignin Mortlock, showed substantial gains in in-vivo digestibility associated with the feeding of whole grain Murray oats to sheep (Rowe and Crosbie 1988). Our studies also showed that the effect of low lignin was enhanced when in combination with low hull silica levels. Whereas lignin level was genetically controlled, the level of silica was influenced mainly by environment.

In the process of this work, we developed a rapid screening test for lignin, using a phloroglucinol reagent, which stained high lignin grains pink. This proved useful in the breeding of low lignin oat varieties.

The above studies and later studies on the implications of feeding low lignin oats to sheep and cattle were recently reviewed (Rowe et al 2001).

Conclusion

Cereal grains are already being extensively used in aquaculture feeds for both functional and nutritional values. Presently there is limited knowledge on the implications of variability in cereal type or quality on their value to this animal feed sector. Most of the information being used by the aquaculture feeds sector is that being extrapolated from pig and poultry industries. However, given that aquaculture feeds are extruded while pig and poultry feeds are steam-pelleted, the implications of cereal quality and variability are likely to be quite different. Furthermore, the developing large-scale aquaculture industries in the near South-East Asian region provide a key target market that has even greater capacity to utilise feed grains and in particular cereals like wheat, which this market does not produce locally.

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Feed grains, Aquafeeds and Extrusion

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Introduction

Modern aquaculture feeds are generally made using an extrusion process (Hardy and Harrows, 2002). This process introduces heat, moisture and mechanical energy to a mixture of raw materials and in the process affects their combined chemistry to change the physical properties of the product produced. The resultant product is usually a more strongly bound and nutritionally superior product (Kaushik, 2001). The improved physical properties are particularly important given the trend of industrial aquaculture using automated feeding systems that can place significant demands on the physical durability of feed pellets.

The importance of feed extrusion in the modern aquaculture field demanded that this technology be considered as part of the program. In addition to this, because of the changes in the chemistry involved in the processing of feeds, it was deemed necessary to gain a good understanding of the influences that different grain varieties had on the products produced using this process. To facilitate this, in 2001 the Department of Agriculture and Food commissioned a laboratory scale extruder, which was set up at Curtin University. This equipment has since been extensively used in the study of extrusion processing on the physical, chemical and nutritional features of feeds for fish. Many of the diets in the program were also prepared using this equipment.

Assessing the impacts of extrusion on grain evaluation

One of the chemical changes that occur during the extrusion process is the gelatinisation of starch within the mixture. This gelatinisation not only assists in binding the pellet, but also significantly improves the digestible energy value of the feed (Figure 1).

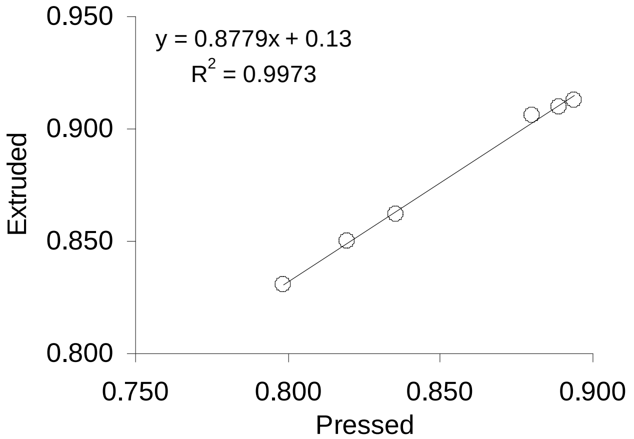

Figure 1. A comparison of the influence of feed extrusion on the digestible energy value of feeds fed to rainbow trout. Each feed was prepared using either extrusion or screw-press technologies.

An assessment of the diet digestibility of energy, protein, dry matter and amino acids showed that it was possible to get a similar or proportional answer on the nutritional value of the grain being assessed whether using either extrusion of screw-press feed processing methods. However extrapolation of the data from the diet digestibilities to calculate the ingredient digestibilities showed that this was only viable for energy and dry matter values, with both protein and amino acids losing correlation between the two feed processing methods. This means that when using screw-press feed processing methods in digestibility studies, that the data is largely only valid for digestible dry matter and energy values of ingredients, but not digestible protein values.

Assessing the impacts of grain variety inclusion on extrusion

Most modern aquaculture feed formulations contain a grain component. Wheat for instance is routinely used as a starch source for binding during the extrusion process. In wheat, both the starch and proteinaceous gluten components of wheat contribute to its functional properties. Studies with a range of feed grains has identified that there is a range of effects produced and these effects vary according the grain variety. However, most grains exerted some functional properties on extruded pellets, however it was unknown as to whether this was an effect of the protein or the carbohydrate components of the product.

Initial testing using commercial testing practices demonstrated that inclusion of lupins in an extruded pellet resulted in high durability of the pellets containing any of the lupin kernel meal products (Figure 2). However, protein concentrates produced from these same lupin kernel meals did not result in any improvements in pellet durability. This supports that it is the carbohydrate component of the lupin kernel meals that is providing most of the binding functionality in extruded pellets.

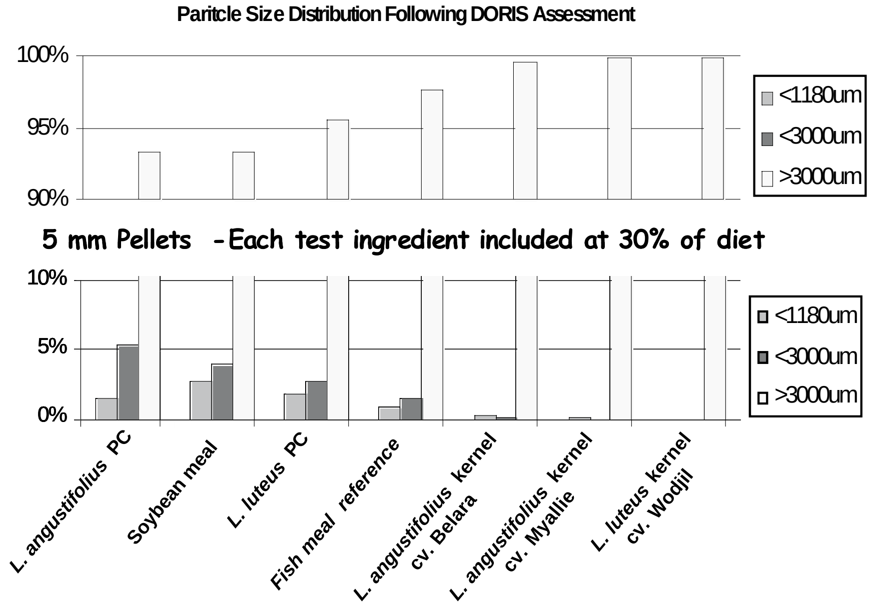

Figure 2. A comparison of the influence of different grain products on the durability (particle size distribution) of pellets following extrusion processing. Treatments with a higher proportion of >3000 um particles are more durable. Treatments with higher levels of <1180 um particles resulted in the most pellet dust following durability testing.

Further extrusion studies were undertaken with a range of feed grains to examine the effect that different inclusion levels had on the physical properties of the pellets produced. The techniques and equipment used were based on those reported from earlier work (Evans, 1999). The different grain meals were included into a reference diet mash at 10%, 20% or 30% inclusion levels. These treatments were then extruded using the same processing conditions (screw-configuration, barrel temperature, water input) in each case. Following extrusion the pellets produced were then subjected to an assessment of their key physical attributes, including: expansion, bulk density, oil absorption, durability, hardness, sink rate, water stability among others. This work built on from earlier studies that examined the effects of the inclusion of *L. angustifolius* kernel meal at 0%, 5%, 10%, 15%, 20%, 25% and 30% levels (Glencross and Sipsas, 2004).

The sink-rate of a pellet is important as a pellet that sinks too fast can result in significantly more feed wastage, while a pellet that floats, although often popular with fish farmers, can result in reduced feed intakes by the fish. The addition of *L. angustifolius* kernel meal to the mash was observed to increase the sink-rate of extruded pellets (Figure 3). However the effect that *L. angustifolius* kernel meal was not that much different to that observed from soybean meal inclusion. In contrast, the inclusion of *L. luteus* kernel meal produced a pellet that had a significantly faster sink rate at all corresponding inclusion levels, except the 30% inclusion level.

The hardness of a pellet is one objective measure of the potential durability of the pellet, an important feature in the modern use of automated feeding systems. The inclusion of *L. angustifolius* kernel meal in the formulation resulted in a significantly harder pellet than that achieved with either soybean meal or *L. luteus* kernel meal (Figure 4). This greater degree of pellet hardness was observed at all inclusion levels. Notably both lupin kernel meals produced harder pellets that those achieved using soybean meal.

Consistent with the observations of sink-rate and pellet hardness, the bulk density was also observed to significantly increase with the addition of *L. angustifolius* kernel meal to the mash (Figure 5). The bulk-density response with *L. angustifolius* kernel meal inclusion was generally linear, but at lower inclusion levels was less than that of the *L. luteus* kernel meal, but greater than that of the soybean meal. The soybean meal consistently produced the least dense pellets in this study. As is to be expected, the pellet density findings are generally consistent with the findings from the sink-rate studies, where the denser the pellet, the faster the sink-rate. A pellet density less than 475 g/L was observed to generally produce a floating pellet.

Pellet expansion can provide an indication of the degree of gelatinisation of the starch within the pellet and also the sink-rate, density and oil uptake potential. Pellet expansion in this study was reduced by the addition of any of the grain varieties studied, with *L. luteus* kernel meal proving to have a much greater overall effect on reducing the expansion of extruded pellets (Figure 6). However with each grain variety a critical inclusion level around the 20% level was observed to limit the expansion the most. Grain inclusion levels above and below this produced pellets with better expansion properties, but still not as great as those achieved in their absence.

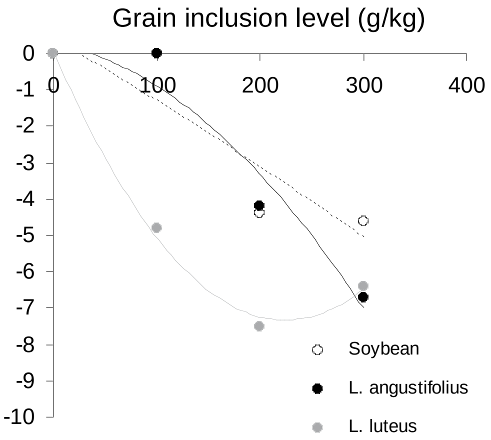

Figure 3. Pellet sink rates (cm/s) as a function of grain meal inclusion.

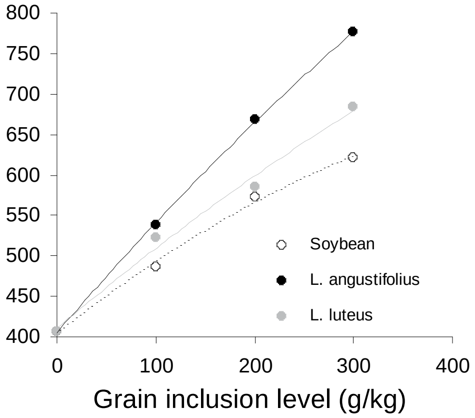

Figure 4. Pellet hardness (g of force to split pellet) as a function of grain meal inclusion.

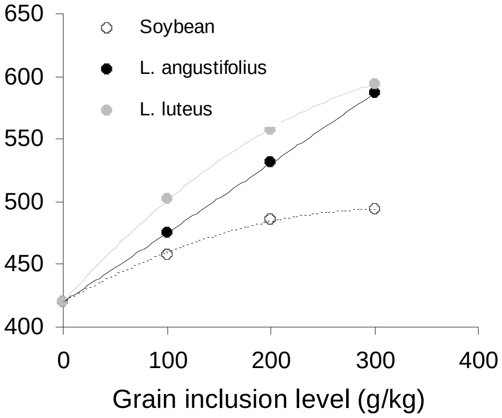

Figure 5. Pellet bulk density (g/L) prior to oil coating as a function of grain meal inclusion.

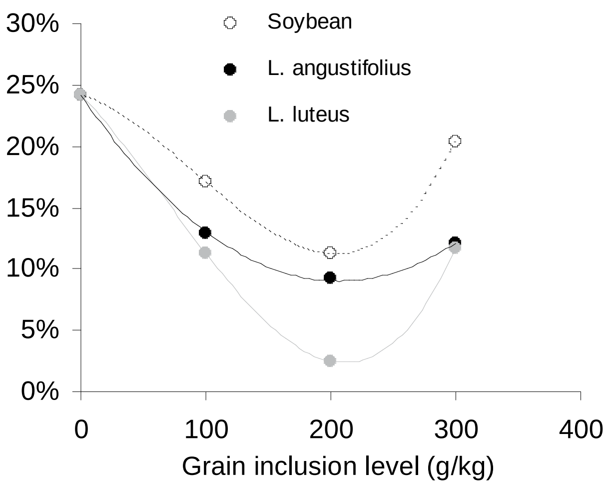

Figure 6. Pellet expansion (%) as a function of grain meal inclusion.

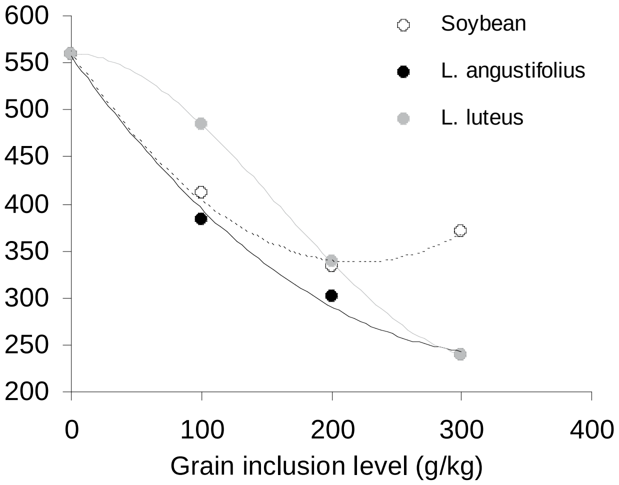

Figure 7. Pellet vacuum oil infusion uptake (g/kg) as a function of grain meal inclusion.

Vacuum infusion is an important process in the production of high-nutrient-density extruded aquaculture feeds. By using vacuum infusion a greater amount of oil can be incorporated into a pellet than by using other feed processing or application techniques (Perez, 2001). In this study the inclusion of any of the grain varieties studied resulted in a reduction in vacuum infusion potential (Figure 7). However the reduction in vacuum oil infusion potential was, at lower grain inclusion levels, not as severe with the use of *L. luteus* kernel meal. At high grain inclusion levels, soybean meal supported higher vacuum infusion potential than both lupin varieties. These observations are somewhat different to those reported by Glencross and Sipsas (2004) who noted that vacuum infusion potential improved with inclusion of *L. angustifolius* kernel meal, up to an inclusion level of 20%. However, it must be pointed out that the two studies used different diet formulation strategies and that in this study the fact that the diet starch level is not kept constant does cause some complications.

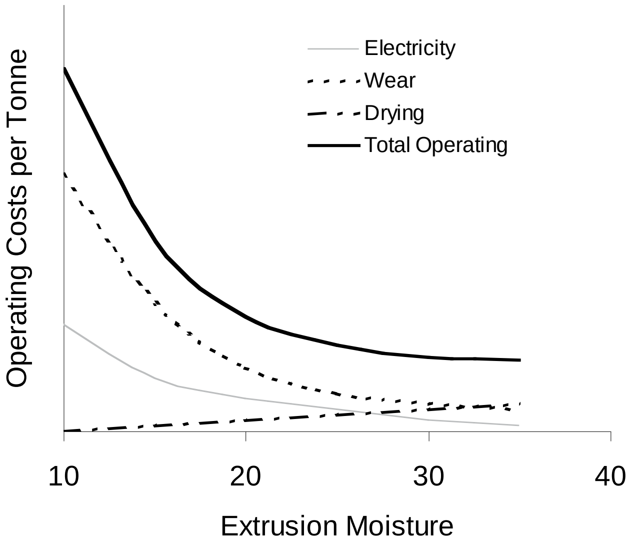

Figure 8. Pellet bulk density (g/L) prior to oil coating as a function of grain meal inclusion. Derived from Rokey (2005).

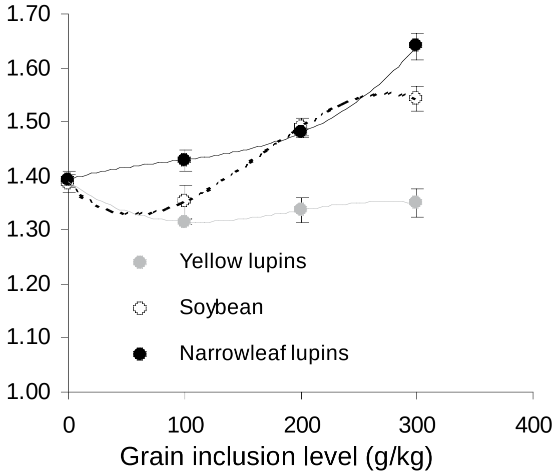

Figure 9. Relative moisture retention as a function of type and level of grain meal inclusion.

The process of extrusion requires the use of certain equipment, which is a significant cost to the process of aquaculture feed production. Furthermore, one of the greater operational costs to an aquaculture feed producer is the depreciation costs associated with the extruder. The rate of deprecation is strongly related to the rate of the wear of the screws and barrel of the extruder. This rate of wear is strongly influenced by the fluidity of the mash flowing through the machine, which in turn is affected by the moisture content of the mash being processed through the machine (Figure 8). Therefore when a feed mash can be made to retain additional moisture in the mash, whilst still maintaining a functional mash for processing, there is substantial potential to reduce the depreciation rate of the extrusion equipment through raw material choice.

A study of the mashes that were used in earlier parts of the extrusion studies identified that the type of grain and its inclusion level also had a significant effect of the moisture retention in the mash (Figure 9). Notable was that the inclusion *L. angustifolius* kernel meal at the lowest level of10% resulted in a significantly higher level of moisture retention than other grain options of soybean meal or *L. luteus* kernel meal. With higher inclusion levels of *L. angustifolius* kernel meal the moisture retention capacity of the mash further improved. The moisture retention capacity of soybean meal also improved at higher inclusion levels and at a 20% inclusion level was equal to that of *L. angustifolius* kernel meal. Increasing inclusion levels of *L. luteus* kernel meal made little improvement in the moisture retention capacity of the extrusion mash.

Improving our understanding the functionality of grains

Because different raw materials have to be combined to create an extruded aquaculture feed it is equally important to understand the potential functional implications associated with the choice of certain raw materials over others. In this sense the practical application of feed manufacturing can be somewhat described as a combination of both an art and a science. The art lies within knowing what can functionally be mixed with what, and what limits there may be on the choice of any one particular raw material. The science being the ability to nutritionally combine the raw materials to provide a combination that will supply the animal's nutrient and energy requirements for growth, without the introduction of compounds that have a deleterious effect on the animal (Kaushik, 2001).

From the work undertaken as part of the program we have identified that different grain varieties have substantially different functional properties and these may or may not have application to aquaculture feeds depending on the specific circumstances. The functional properties also vary not only with grain variety, but also inclusion level. That many of the observation functional properties are not linear responses suggests that there are possible interactive effects that affect this feature of raw material choice. The use of technology such as rapid viscosity analysis (RVA) has in relationship to extrusion processes shown some potential to predict the gelatinisation potential of certain raw materials and mixes. The RVA technology has also shown some capacity to predict properties of the final product (Glencross and Sipsas, 2004).

While the strategy used in the studies in this research program represents one approach option, it is by no means the only approach. Different strategies, including the use of nutrient balanced diets are another approach that also has substantial application (Glencross and Sipsas, 2004).

Future work in this area will need to consider an even greater variety of grain options and in particular a need to focus on the functionality that different cereal varieties offer and how these can be used to improve product options for the aquaculture feed sector (Thomas, and van der Poel, 2001).

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The future of feed grain production in Australia - the pulse outlook

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Introduction

Annual feed grain consumption in Australia is currently in the order of 10 million tonnes and is increasing. Stockfeed manufacturers use Australian grown cereals, pulses and oilseed meal and imported soybean meal as sources of protein and energy. Purchasing decisions for these ingredients are influenced by the nutritional and functional requirements of required feed formulations, cost and continuity of supply, and mill storage availability (Parkes, 2006). Narrow-leafed lupin (NLL) (*Lupinus angustifolius*) and field pea (*Pisum sativum*) are the pulses most used in Australia, with some use of faba bean (*Vicia faba*), albus lupin (*L. albus*) and vetch (*Vicia sativa*).

A major limitation to the use of pulses in stockfeeds in Australia is the geographic displacement of supply and demand (Tables 1 & 2). There is a large production of NLL and an expanding production of field pea in Western Australia (WA), which vastly outstrips the needs of the local feed industry. In contrast, there is a small production of feed pulses in eastern Australia, particularly in New South Wales (NSW) and Queensland, relative to the size of the domestic feed industry. Consequently Australia imports up to 300,000 tonnes of soybean meal annually. Freight rates and quarantine barriers (in the case of lupins) currently make the movement of grain from WA and South Australia (SA) to the other states difficult and expensive.

Table 1. Australian livestock numbers (‘000) by region, 2003-04.

	Beef Feedlot	Dairy	Broilers	Pigs
Qld central	194	41	134	999
Qld south	640	89	63,537	1,467
NSW northern	385	80	35,935	513
NSW Hunter	3	36	80,801	257
NSW south east	8	56	56,129	506
NSW south west	350	46	29,462	1,681
Vic north east	1	359	103,948	122
Vic north west	118	433	7,545	2,103
Vic south west & SA lower SE	9	381	20,702	260
SA	59	75	57,910	1,776
WA	89	59	41,160	1,204

Adapted from Hafi and Connell (2003).

Table 2. Australian production ('000 tonnes) of main feed ingredients by region, 2003-04.

	Wheat	Barley	Sorghum	Lupin	Field pea	Faba bean	Canola meal
Qld central	25	7	509				
Qld south	42	96	690				
NSW northern	1,009	318	735	[B] 5	8	4	220
NSW south east	66	2	0	[A] 2	0	0	14
NSW south west	755	312	4	[A] 11	22	12	0
Vic north east	297	48	1	[A,B] 10	11	6	120
Vic north west	1,431	434	2	[A] 16	121	71	0
Vic south west & SA lower SE	188	49	0	[A] 6	2	1	0
SA	1,655	1,241	0	[A] 55	166	162	22
WA	5,549	1,124	0	[A] 850	94	9	16
Total	10,929	3,631	1,945	947	424	268	392

Adapted from Hafi and Connell (2003); [A] = *L. angustifolius*; [B] = *L. albus*.

Pulse composition

Field pea and faba bean are true pulses like chickpeas and lentils with a moderate protein content and considerable starch. NLL and Albus lupin on the other hand are similar to soybean having high protein and virtually no starch. But where soybean has a high oil content lupin is high in fibre. Two other lupin species are being researched as a potential new feed grain for Australia. The yellow lupin (*L. luteus*) has been widely utilised in northern and Eastern Europe and has shown potential for use on infertile acid soils in Australia. The pearl lupin (*L. mutabilis*), a native of the Andean highlands of South America has yet to be developed anywhere for modern agriculture but has higher oil as well as protein (Table 3).

Table 3. Typical whole seed analysis of Australian peas, beans and lupins (% as received basis).

	Field pea	Faba bean	Narrow-leafed lupin	Albus lupin	Yellow lupin	Pearl lupin[D]
Crude protein	23.00	24.00	32.00	36.00	38.00	43.00
Total lipid	1.10	1.20	5.80	9.10	5.60	15.00
Starch	42.00	44.00	1.00	2.00	1.00	1.00
Crude fibre	6.00	8.00	15.00	11.00	16.00	7.00
Soluble fibre[A]	21.00	18.00	42.00	32.00	35.00[B]	-
Methionine	0.19	0.18	0.22	0.24	0.27	0.28
Cysteine	0.34	0.32	0.42	0.49	0.88	0.68
Lysine	1.61	1.46	1.46	1.58	2.07	1.49
Tryptophan	0.18	0.15	0.32	0.37	0.32	0.30
Oligosaccharide	3.50	2.70	4.10	6.60	8.90	5.00
Phytate	0.60	-	0.50	0.60	0.90	-
Tannin	1.0C	1.00	0.30	0.40	0.30	-

Adapted from Petterson, Sipsas & Mackintosh (1997); [A] Soluble fibre includes non-starch polysaccharide and oligosaccharide; [B] Flis *et al.* 1999; [C] Dun type; [D] M. Sweetingham, J. Clements & S. Sipsas (unpublished data).

Pulse usage in feed rations

Pulses are rarely fed as the sole component of a ration. As an energy source they compete with cereals and as a protein source they compete with oilseed meals. It is their capacity to complement other feed components to achieve overall nutrient balance at minimal cost that determines their value. Thus the protein/energy balance, amino acid profile and lack of anti-nutritional factors often touted as positives for pulses are not of overwhelming importance (Edwards, 2004). However, pulses can provide functional benefits in feed extrusion or pelleting that can add additional value.

Field pea is the preferred pulse for the poultry and pig industry with low fibre, good energy, good protein digestibility, high lysine availability and a starch level that assists steam pelleting. Whilst faba bean is well regarded as a pig and poultry feed ingredient, some Australian data shows they have a lower energy yield and amino acid availability compared to field pea. It is likely that this is related to the tannin content and perhaps the nature of the fibre fraction (Edwards, 2004).

NLL is the most valued pulse by the ruminant markets as a protein and energy source, where they are preferred over field pea and faba bean because of their low acidosis risk and rumen fermentation properties (Edwards & van Barneveld, 1998). The high fibre content limits their inclusion rate as whole grain in pig and poultry rations. Because of their high fibre content, lupins are typically de-hulled before being fed to pigs, poultry and fish. As the cellulose-rich hull makes up nearly 25% of the weight of the seed the resulting kernel meals have significantly higher protein (Table 4).

Table 4. Typical composition (% as received basis) of lupin kernel meals compared to de-fatted soybean meal.

	Soybean meal 48	Narrow-leafed lupin	Albus lupin	Yellow lupin	Pearl lupin
Crude protein	48	41	44	52	49
Total Lipid	2	7	11	7	17
Polysaccharide	23	29	21	11	–
Oligosaccharide	11	6	8	12	6

Adapted from Smith (2005)

With increasing pressure on global fishmeal stocks the demand for vegetable protein to replace fishmeal in aquaculture diets has stimulated interest in the potential of lupin. The salmonid and prawn feed markets have been identified as prospective markets for lupin kernel meal. The high protein requirements for these diets reduce formulation flexibility and increase the value per unit protein or energy (Glencross 2005). Internationally, these markets consume about 3.6 million tonnes of feed each year but aquaculture accounts for only 1% of stockfeed use in Australia.

L. albus has higher oil and less hull proportion with the same desirable features for ruminant feed as narrow-leafed lupin. It has good metabolisable energy for poultry but has been shown to reduce feed intake in pigs at 15% inclusion rates or higher. The reason for the effect on pigs is not fully resolved, but alkaloids, manganese and amino acids levels have been discounted. It appears likely to relate to the non-starch polysaccharide and/or oligosaccharide component, although this is not obvious from the proximate analysis compared to NLL and yellow lupins, which do not have the same problem.

Yellow lupins have been commercially used in Russia, Poland and Germany for ruminants, pigs and poultry for many years whilst only small volumes have been available in Australia. The higher crude protein, which is richer in lysine and sulphur amino acids than NLL, offers significant advantages to monogastrics and particularly to aquaculture. The extent of the advantage becomes evident upon de-hulling as yellow lupin kernel meals exceed the soybean meal benchmark (Table 4). The high protein requirements of salmonid and prawn diets have been identified as a potential niche for yellow lupin. In rainbow trout and red seabream the protein digestibility of yellow lupin kernel meal is better than that of the soybean meal, with a similar overall digestible dietary energy (Glencross, 2005).

The grain composition of the pearl lupin is very similar to soybean making it a grain that could be crushed for its oil leaving a high protein meal. Very limited feed utilisation data is currently available. Protein digestibility of pearl lupin has been shown to be similar to other lupin species in rainbow trout (B. Glencross, unpublished). Pig feeding trials are planned for 2008 by the Pork CRC.

Pulse Breeding in Australia

Pulse breeding programs in Australia primarily aim to increase yield and improve disease resistance to increase the profitability for grain growers.

The grain quality targets for Australian field pea breeders are directed at the human consumption markets in the Indian subcontinent where there is potential for price premiums. The focus is on improving grain size, shape, uniformity and seed coat properties for the yellow split pea market. In Europe, North America and China zero tannin types (eg white peas) are preferred on the basis they improve protein digestibility. This factor has not been recognised in Australia and as we gain export food market advantage from dun peas, breeding for zero-tannins types remains a low priority.

The grain quality targets for Australian faba bean breeders are orientated to the Middle East food market where the important parameters are hydration capacity, seed size and appearance. Breeding low tannin varieties has been achieved overseas. The Australian program has low tannin genes in a reasonable agronomic background and is putting some resources into developing this material. Unfortunately the European experience has been that the price premiums for low tannin varieties do not offset their slightly lower yields.

Whilst small improvements in protein and amino acid profiles are possible for field pea and faba bean this would be of minimal economic significance to most stockfeeders and are therefore not a sensible breeding investment.

NLL breeding is exploring possibilities to increase seed protein from 31 to 36% and to reduce hull proportion from 25 to 20% without compromising yield, which would increase their value for de-hulling for both feed and food uses. Progress in protein has been made with the variety Coromup released in 2006 (Table 5). Coromup yield is 6-10% lower than Mandelup but is equal or better than all other lupin varieties under Western Australian conditions.

Table 5. Typical protein and oil levels of modern narrow-leafed lupin varieties grown under the same conditions.

	Crude protein (N x 6.25)%	Oil %
Belara	31.6	6.1
Mandelup	32.3	5.7
Kalya	33.2	5.4
Merrit	33.6	5.6
Quilinock	33.6	5.2
Coromup	34.5	5.8

Average protein levels for a given variety can vary with region and season but the ranking of the varieties remains relatively consistent. Research is continuing to understand discrepancies between crude protein (N x 6.25) and digestible protein levels in lupin varieties as this has important ramifications for monogastrics and aquaculture. GM technology can be used to increase the proportion of sulphur amino acids in the protein but the current cost of commercialising this technology exceeds the benefits.

There are currently no resources to breed for improved grain quality in *L. albus*.

Yellow lupin production is minimal at present as the varieties available are based on types from northern Europe, which have proven poorly adapted to Australian conditions. Research into the genetic improvement of yellow lupins is therefore focussed on improving yield potential and aphid resistance whilst maintaining current grain protein levels. Varieties significantly better than cv. Wodjil are five years off.

A small pearl lupin breeding project has commenced in Australia and a variety could be released as soon as 2010, but it would likely be only suitable for high rainfall well drained soils. The breeding priorities are to increase yield potential and disease resistance under Australian conditions.

Substantially altering grain composition by either conventional breeding or using GM technology is both difficult and expensive. It may also be counter-productive because of trade-offs in yield or important agronomic characteristics. An important overarching strategy may be to enhance regional adaptation to help boost production of the different species close to the relevant feed demand.

Production Outlook

The pulses discussed in this paper are grown over winter in annual rotation with winter cereals, canola or annual pastures. In any given paddock they are typically grown every 3rd or 4th year as a precaution against build-up of disease. In this sense they complement cereals and canola because they fix atmospheric nitrogen and have a different disease spectrum. However, grain growers planting decisions are influenced by seasonal conditions and increasingly by price outlook.

Field pea production has traditionally been highest in South Australia and the Wimmera and southern Mallee regions of Victoria. In recent years production has increased in WA, particularly north of Esperance. The advent of higher yielding and more erect varieties (such as cv. Kaspa) which make harvesting easier is increasing popularity with growers in WA. Small areas are grown in NSW, mainly in the south-west. The crop has good adaptation across a wide range of soils and is relatively drought tolerant. It has potential to expand in all states and provide reliable local supply. However, field pea competes with chickpea and lentil, which receive price premiums in the food markets, and faba bean, which perform better in higher rainfall environments.

Faba bean production is greatest in South Australia with expanding production in Victoria and New South Wales. Compared to other legume crops it is usually more profitable in medium and higher rainfall zones now that disease resistant varieties (cvs. Farah and Nura) have been developed. Potential for further expansion of production will be influenced by competition from other pulses and canola. It maybe desirable for faba bean producers in northern NSW to more directly target stockfeed rather than food grades owing to proximity to significant domestic pig and poultry markets.

NLL are likely to remain the biggest pulse crop in Australia. The crop is best adapted to deep acidic sandy soils with the majority of production based in the northern cropping zone of WA. NLL was widely grown under minimum tillage in continuous 1:1 rotation with wheat in WA in the 80s and 90s. WA produced 1.6 million tonnes of NLL in 1996. Herbicide resistant weeds have emerged and prices have been driven down by the increasing global soybean meal supply. Consequently the area sown has declined in recent years, and production affected further by a run of dry seasons. It is predicted that in the short to medium term NLL will contract to the most suitable environments in WA and production should stabilise at 800,000 - 900,000 tonnes given relief from drought conditions.

Production is also consistent on suitable soils on the lower Eyre Peninsula and the SA and the Victorian Mallee regions. Production in NSW has fluctuated quite dramatically over the past 15 years but seems most reliable in the southern medium to low rainfall zones. A potential new variety WALAN 2224 promises to more suitable for production in southern Western Australia and possibly also parts of South Australia.

A large scale de-hulling plant has been built in Perth, WA by Australasian Lupin Processing Ltd. with the potential to process up to 200,000 tonnes per annum. Only a small proportion of the kernels are likely to be used for human consumption in the near term leaving substantial quantities for the feed industries.

The disease anthracnose wiped-out *L. albus* in WA in 1996 and it is yet to recover. A partially resistant variety, cv. Andromeda, was released in 2006 and should result in some replanting in lower disease risk areas. Anthracnose is not present in cropping regions of NSW where it is grown in the higher rainfall parts of southern NSW and in northern NSW where virus has been a problem in NLL. The crop services a 25-30,000 tonne human consumption market in the Middle east which pays growers a significant premium over its value to the stockfeed industry. Better varieties (cvs. Luxor and Rosetta) have recently been released in NSW which should result in an increase in production which will saturate the food market and see more grain become available for feed. Market development maybe better focused on ruminants, poultry and possibly aquaculture given the likely technical difficulty and limited resources available to overcome the problems with pigs.

The potential for yellow lupin production in Australia is greater than both Albus and pearl lupin based on our knowledge of soil types and the market opportunities. Yellow lupin can potentially grow on all soils that NLL currently occupy. The extent to which the yellow lupins might expand will depend on the ability to breed better varieties. However, in the short to medium term production based on cvs. Wodjil and Pootalong are likely to be limited to a small number of growers in WA with production contracts for defined markets. Currently we are seeing a shift of interest in yellow lupins from the low rainfall eastern wheatbelt to the southern high rainfall zones.

Looking further to the future, opportunities to manufacture protein concentrates or isolates from lupin or field pea could play a role in high performance feeds and pet food. A viable business case to go down this route would require finding markets for the by-products, dietary fibre for lupins and starch for field pea.

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Further information

www.lupins.org

http://www.fish.wa.gov.au/docs/op/op024/index.php?0306

Making Feed Grains More Valuable – Protein Concentrate Production

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Introduction

Soy protein preparations have been used widely in the food and feed sectors primarily due to their high protein content, good nutritional value, and lower price compared with animal proteins, soybeans were the first source of protein concentrates and isolates as food ingredients, and more recently feed ingredients.

Over the past few years we have investigated the production of protein-enriched products (concentrates and isolates) from lupins. To assess a new ingredient's potential to be adopted, the prospective protein levels at which it will to be cost-effective to both produce and to use need to be determined

Lupins as starting material for Concentrates and Isolates

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

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

Species	***L. angustifolius***		***L. albus***		***L. luteus***		***L. mutabilis***	
	Seed	**Kernel**	**Seed**	**Kernel**	**Seed**	**Kernel**	**Seed**	**Kernel**
Seed Coat	24.0	0.0	18.0	0.0	27.0	0.0	16.0	0.0
Moisture	9.0	12.0	9.0	11.0	9.0	12.0	9.0	12.0
Protein	32.0	41.0	36.0	44.0	38.0	52.0	44.0	52.0
Fat	6.0	7.0	9.0	11.0	5.0	7.0	14.0	17.0
Ash	3.0	3.0	3.0	4.0	3.0	4.0	3.0	4.0
Lignin	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Polysaccharides	22.0	28.0	17.0	21.0	8.0	10.0	9.0	10.0
Oligosaccharides	4.0	6.0	7.0	8.0	9.0	12.0	5.0	6.0
Minor Components	0.5	1.0	0.6	1.0	0.9	1.0	1.0	1.0
Total sum	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

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

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

Lupin concentrates; effect of species and conditions on concentration capacity

The process utilised to produce the lupin concentrates mirrors the commercial process employed to produce soy protein concentrates (Figure 1) by washing the flour with Ethanol (~ 70%), then decanting the supernatant and drying the residue which now has an elevated protein level (Protein Concentrate).

The concentration capacity for Narrowleaf lupin (NLL; *Lupinus angustifolius*) (var. Mandelup) and Yellow lupin (YL; *Lupinus luteus*) (var. Wodjil) grown during the 2003 season was analysed using the standard "soy concentration method" (Figure 2). In NLL approximately 23 per cent of the starting weight, consisting of oligosaccharides, some protein (N x 6.25), and some fat (mainly the phospholipids) was eliminated (Table 2). In YL approximately 29 per cent of the starting weight, consisting predominantly of oligosaccharides, some protein (N x 6.25) and some fat is eliminated (Table 3).

An important point to note is that the Soy Industry has set the benchmark at 65 % protein (dry basis) for Protein Concentration product to classify. According to the results presented only the Wodjil cultivar reached the minimum protein concentration needed to classify in this category.

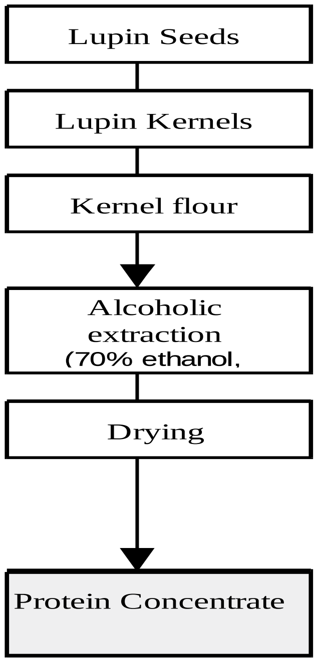


Figure 1. Schematic flow chart for the production of lupin concentrate.

Table 2. Distribution of Narrowleaf Lupin var. Mandelup, kernel flour components via the concentration process (Figure 2).

NLL var. Mandelup Component	100 g raw wt (g)	Concentrate		Discarded material wt (g)
		wt (g)	% of product	
Protein	46.0	40.9	53.1	5.1
Fat	8.0	6.5	8.4	1.5
Ash	3.5	2.0	2.6	1.5
Lignin	0.7	0.7	0.9	0.0
Kernel polysaccharides	32.8	27.0	35.0	5.8
Oligosaccharides	8.0			8.0
Minor components	1.0			1.0
Total sum	100.0	77.1	100.0	22.9

Table 3. Distribution of Yellow lupin var. Wodjil, kernel flour components via the concentration process (Figure 2).

YL var. Wodjil Component	100 g raw wt (g)	Concentrate		Discarded material wt (g)
		wt (g)	% of product	
Protein	57.5	51.2	71.7	6.3
Fat	8.0	6.5	9.1	1.5
Ash	4.5	3.0	4.2	1.5
Lignin	0.7	0.7	1.0	0.0
Kernel polysaccharides	14.0	10.0	14.0	4.0
Oligosaccharides	14.0			14.0
Minor components	1.3			1.3
Total sum	100.0	71.4	100.0	28.6

Optimisation

To optimize the concentration process a number of treatments were trialled using the two lupin kernel flours. The treatments included the washing of all flours at 65°C and 25°C using a range of ethanol concentrations (0-60%). Figure 2 shows the protein concentration achieved from the two lupin kernel flours. It can be seen that there is almost a 15% difference in end protein concentrations at the maximum levels achieved. It is interesting to note that the difference in protein of whole grain NLL (30%) vs. YL (38%) is 8% however when subjected to concentration regime the difference widens to 15%.

The yields between the preheated and unheated Wodjil flour for the water (0% EtOH) treatment showed a 7 per cent increase in yield for the preheated treated flour (Figure 4). This may be due to higher protein solubility of the unheated flour resulting in protein being washed out, whereas the proteins in the heat-treated flour would be denatured and presumably less soluble and retained in the concentrate; resulting in higher yields. However the decrease in yield and protein recovery of the preheated flour, at the 20 per cent ETOH was unexpected (Figures 3 and 4).

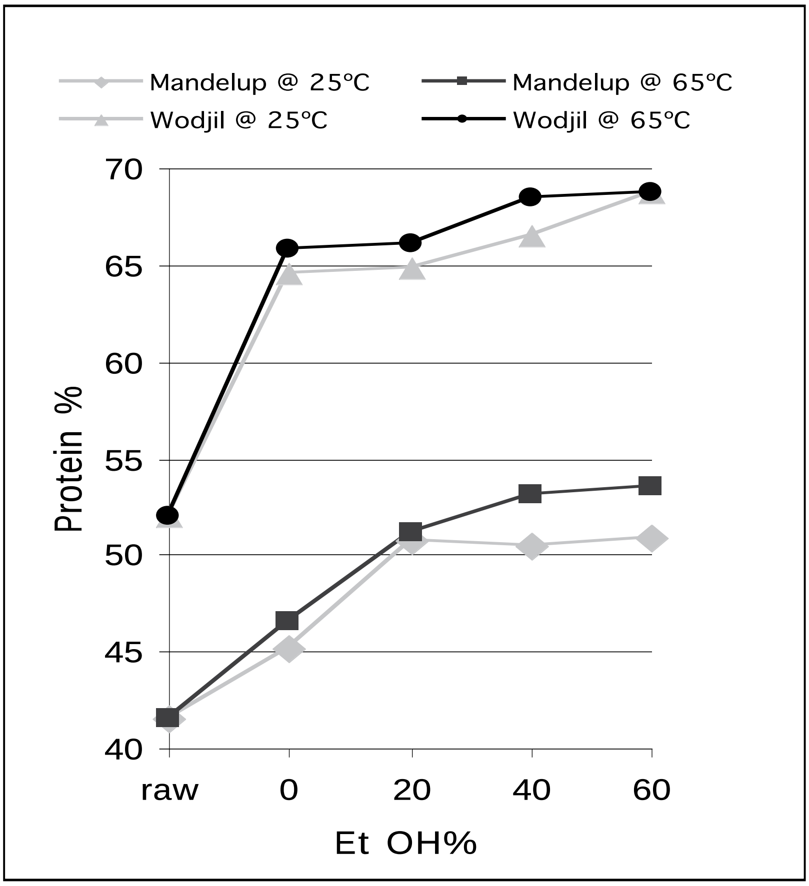
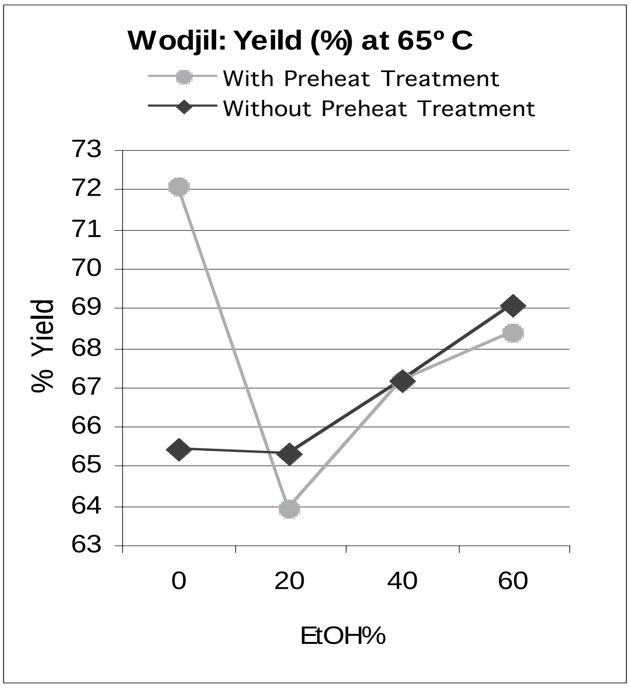


Figure 2. Protein concentration achieved from Mandelup and Wodjil lupin kernel flours with and without preheat treatment washed with a range of ethanol concentrations (0-60%) at 65°C and 25°C.



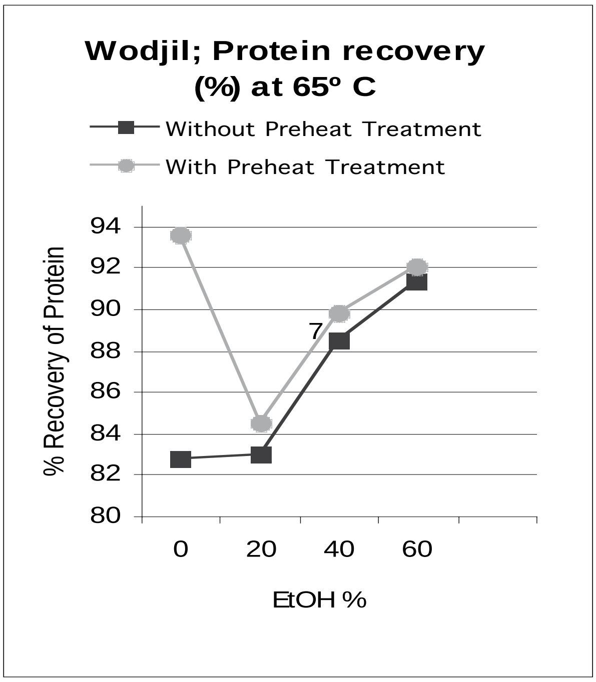


Figure 3. Yield achieved from two Wodjil lupin kernel flours with and without preheat treatment washed with a range of ethanol concentrations (0-60%) at 65°C.

Figure 4. Protein recovery achieved from two Wodjil lupin kernel flours with and without preheat treatment washed with a range of ethanol concentrations (0-60%) at 65°C 60%) at 65°C.

This was possibly due to an increased solubility of a particular protein in Wodjil under those conditions. Curiously the same pattern is observed with the NLL preheated flour at 20 per cent EtOH. With increasing ethanol concentration the proteins are becoming increasingly less soluble, leading to a greater yield.

The protein recovery between the preheated and unheated flour for water (0% EtOH) treatment showed a reduction in protein recovery of approximately 11 per cent for unheated treatment (Figure 4). This may be attributed to high protein solubility of the unheated flour resulting in protein being washed out from the two lupin kernel flours.

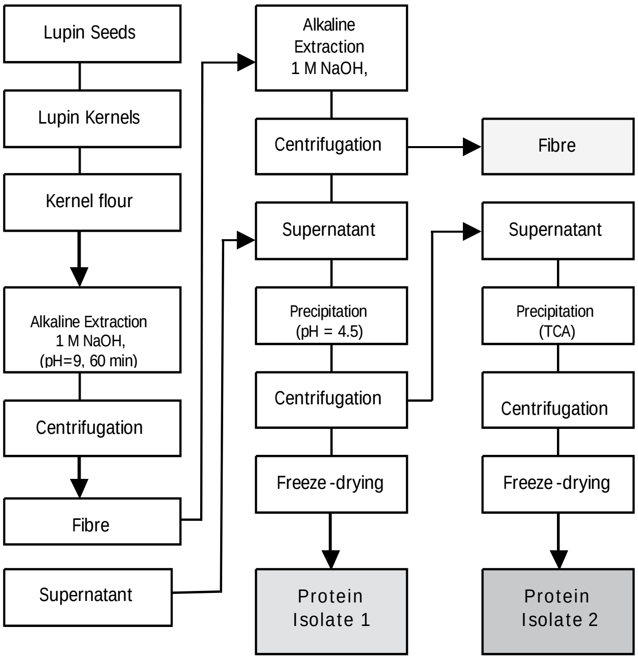


Figure 5. Flow diagram for the production of the lupin fibre, Protein Isolate 1 and Protein Isolate 2.

Protein isolates

In developing and applying a process for NLL and YL, the commercial soy process was used as a model. Most isolated soy protein products are produced by slurrying flakes/flour with water, then using an alkaline extraction (pH 9), separating the insoluble material from the water-soluble protein then precipitating the protein (Isolate 1) with acid (pH 4.5) (Figure 5). When this process is applied to lupin there is a component of the lupin protein that is still soluble at pH 4.5, which needs ultra-filtration for collection (Isolate 2). Isolate 2 has unique functional properties and would represent a significant protein loss (as well as creating a waste problem) if discarded. In the case of lupin the insoluble material, the Fibre is a valuable by-product, which has useful functional properties (Evans and Htoon, 1996) and health benefits (Archer, et al., 2004; Johnson, et al., 2003 and Hall, et al., 2005).

Approximately 75 per cent of the original weight was recovered as Fibre, Lupin Isolate 1 and Lupin Isolate 2. Lupin Isolates 1 and 2 accounted for 36 per cent of the original weight and 87 per cent of the original protein was recovered in all three fractions (Table 1). The basic compositions of these three fractions are:

- Fibre: pectin like polysaccharides made up of galactouronic acid and rhamnose chains (Cheetam, et al., 1993; Evans, 1994).
- Protein Isolate 1: globulin proteins (α and β conglutins).
- Protein Isolate 2: albumin proteins and δ conglutins.

The material lost consists of nitrogen mostly in a non-protein form.

Table 4. The distribution of protein, fat and carbohydrates in the Fibre, Protein Isolates 1 and 2 extracted via 'standard extraction' from Narrowleaf Lupins.

NLL var. Myallie		**Fibre**		**Protein Isolate 1**		**Protein Isolate 2**		**Material lost**
Kernel flour (dry basis)	WT(g)	WT(g)	% of product	WT(g)	% of product	WT(g)	% of product	WT(g)
Protein	46.0	8.0	20.4	27.0	91.5	4.5	75.0	6.5
Fat	8.0	1.5	3.8	1.5	5.1	0.5	8.3	4.5
Ash	3.5	2.0	5.1	1.0	3.4	0.2	3.3	0.3
Lignin	0.7	0.7	1.8				0.0	0.0
Kernel polysaccharides	32.8	27.0	68.9			0.8	13.3	5.0
Oligosaccharides	8.0							8.0
Minor components	1.0							1.0
Total sum	100.0	39.2	100.0	29.5	100.0	6.0	100.0	25.3

Species effect on isolates

The extraction efficiency for 4 Lupin species; *Lupinus angustifolius* (Narrowleaf Lupin - NLL), *Lupinus albus* (Albus), *Lupinus luteus* (Yellow Lupin -YL), and *Lupinus mutabilis* (Mutabilis) is reported in Table 7. Overall the protein recoveries are similar (~85% to 90%) for all species with the exception of Albus, which is about 10 per cent lower. YL and Mutabilis would be excellent protein sources for the production of Protein Isolates given the high kernel protein however a defatting step would have to be introduced for Mutabilis.

Table 5. Mass balance data of the protein extraction efficiency of four lupin species.

Species	**Coarse Kernel meal**	**Fibre**		**Protein Isolate 1**		**Protein Isolate 2**		**Fibre + Isolates 1 + Isolate 2**		
% CP		WT Yield %	% CP	WT Yield %	% CP	WT Yield %	% CP	Weight recovery %	CP (%) recovery	True Protein
NLL (Myallie)	42.1	52.7	30.8	19.3	88.1	5.0	73.8	77.0	87.7	36.9
Albus (K. Mutant)	50.0	46.3	30.2	24.5	88.6	4.4	74.9	75.2	78.0	39.0
YL (Wodjil)	51.6	43.0	39.1	25.3	88.6	5.8	74.5	74.1	84.4	43.5
Mutabilis	50.3	43.7	41.8	28.6	83.1	3.1	72.3	75.4	88.0	44.3

Lupin isolates: Optimisation of extraction efficiency

The degree of protein exhaustion of the Fibre residue is a key point in driving the efficiency of the extraction process. The optimum scenario would be to separate the protein from the Fibre as cleanly as possible. A 100 percent separation is not possible as there are proteins chemically bound within the Fibre fraction.

To optimise the extraction process a number of treatments were trialled using NLL var. Myallie grown at Wongan Hills in 2001. The treatments were in three groups (Table 6). Treatments in group A used coarse Myallie kernel meal as a starting material. Treatment group B used finer Myallie kernel meal and treatment group C analysed the effect of kernel meal particle size.

Table 6. Protein extraction efficiency of NLL var. Myallie, (Wongan Hills, 2001) using different treatments.

ID	NLL var. Myallie	Kernel Meal	Fibre		Isolate 1		Isolate 2		Fibre + Isolates 1+ 2	
	Treatments	% CP	WT Yield %	% CP	WT Yield %	% CP	WT Yield %	% CP	WT Yield %	% CP recovery
Treatments group A: Coarse kernel meal particle size; 23 per cent 500 μm										
C	Control	42.1	52.7	30.8	19.3	88.1	5.0	73.8	77.0	87.7
A1	Acid wash	42.1	52.6	31.0	18.9	90.1	2.4	77.3	73.9	83.6
A2	Overnight Soak	42.1	41.5	22.7	15.7	89.9	6.1	75.3	63.4	66.9
A3	Overnight Freeze	42.1	45.9	28.5	17.2	88.4	6.0	72.6	69.1	77.5
A4	70 Degree Celsius	42.1	51.1	32.5	18.8	83.4	6.3	78.5	76.2	88.4
A5	pH 12	42.1	51.0	28.6	22.6	86.2	4.3	76.5	77.9	88.7
Treatments group B: Fine kernel meal particle size; 83 per cent < 500 μm										
C	Control	42.1	46.7	25.7	23.0	91.3	6.0	74.8	75.7	91.7
B1	Pectinase (2 h)	42.1	47.4	23.7	14.3	89.0	11.0	80.4	72.7	77.9
B2	Pectinase (16 h)	42.1	42.1	21.8	14.6	85.8	10.6	74.7	67.3	70.4
B3	Acetate buffer	42.1	41.0	18.8	18.6	82.2	14.6	71.8	74.2	79.5
B4	pH 9 (16 h)	42.1	35.8	15.7	29.4	81.7	12.3	75.3	77.5	92.5
Treatments group C: Kernel meal particle size variation										
C1	Coarse	42.1	53.0	30.8	19.3	88.1	5.0	73.8	77.3	87.9
C2	Fine	42.1	49.0	25.0	23.3	86.2	7.3	78.4	79.6	90.4
C3	Flour	42.1	32.0	14.4	30.3	91.2	8.0	75.6	70.3	90.9

Results / Discussion

Treatments in group A investigated different methods of releasing proteins from the Fibre without using (costly) mechanical energy to produce flour. A coarse meal starting material was utilised and various 'wet softening' methods were trialled including an acid-prewash (A1), overnight soaking (A2), freezing (A3), pre-heating (A4) and overnight soaking with elevated alkaline extractions (A5). As shown, the preheating and overnight extraction at pH 12 had no effect. The treatments involving prolonged soaking (including the freezing treatment) of the flour in water did produce Fibre fractions with lowered protein levels but these were not converted to increased yields in the protein isolates. Notably both these treatments resulted in significant yield losses and lowered protein recoveries. This suggests that at pH 5 (the pH of lupin flour in water), there is a native protease which is active and it appears that the protein was degraded as the yield of Isolate 1 was reduced. Hence the total protein recovery was also reduced. This protease must be inactivated by higher pH values as this degradation was not evident in the overnight extraction at pH 9 (B4) (Table 6).

As it seemed that the 'wet softening' approach was not creating significant advances, it was accepted that a finer particle size was a crucial variable in the process. Treatment group B utilised a 'fine meal' and investigated the use of the enzyme pectinase as a means of disrupting the polysaccharide chains in the Fibre adequately to release the protein bodies. It also included an extended (16 h) extraction at pH 9. As particle size affects kinetics, this treatment would clearly indicate if the 'fine meal' should be finer still. Both pectinase treatments only slightly lowered the residual protein left in the fibre fractions but both significantly lowered the yield of protein Isolate 1 and significantly increased the yield of Isolate 2. The yield and protein recovery losses resulted in both treatments with the 16 h pectinase treatment the most effected. Both pectinase treatments were conducted in acetate buffer and the buffer (minus pectinase) treatment presented unexpected results (Table 6). The acetate buffer affects the extraction significantly, as the Fibre protein is lowered, the protein yield of Isolate 1 is lowered (although not as much as the pectinase treatments) and the protein yield of Isolate 2 is increased by over 200 per cent. However there is a total yield loss and lowered protein recovery (Table 6).

Treatment group C – investigated the effects of kernel meal particle size, which a critical variable in the efficiency of extracting maximum protein away from the fibre residue as indicated by the results. Fibre protein levels decreased as the particle size of the starting material decreases from 30.8 per cent to 14.4 per cent. Concomitantly there was an increase in yield of the protein Isolate fractions (1 and 2) from 24.3 per cent to 38.3 per cent as protein is released from the Fibre (Table 6).

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Grains Research and Development Corporation (GRDC) and Aquaculture

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Summary

The GRDC receives a levy from grains produced in Australia. The main income for the GRDC is obtained from wheat and barley. The GRDC has supported an aquaculture component in its project portfolio since 2000. These have included comparisons of Lupins to Soybeans for protein concentrates and isolates, in salmon and rainbow trout. Two critical events have occurred that will alter the funding provided to the aquaculture component of the GRDC project portfolio. These are the way the GRDC assesses and prioritises its projects and the drought.

GRDC Changes

Each of the GRDC lines of business use a ranking system for the projects that are received. A project's rank is based on a number of factors, but increasingly, emphasis is being placed on what is the benefit to grain growers and what does the business case look like. In aquaculture, the flow of benefit back to the grain grower is not immediately apparent. Nor are the market drivers apparent that make the farmer investment into crops for aquaculture apparent. However, if the market drivers can be established then there are opportunities both in the increased production and reliance on aquaculture as world fish stocks are reduced. As is the case in all the domestic livestock feed grain sectors, for these drivers to occur the grain qualities need to be better aligned with market needs, which in this case seem to be nutritional value and functional properties. However, I think that at the moment the grain volumes required in the aquaculture area are not significant enough to create a strong business case. This may well change in the future as I have insinuated. For the international markets I think the case is different again. High volumes but very much more cost sensitive, does not create a driver to invest in this area.

Having said this, if competitive advantages in particular areas can be found, whether it is in the grains or the growth of the domestic aquaculture industry, then the business case should be reassessed.

The drought

The drought has had a significant impact to the grains industry and as a consequence the income from the grain levy to the GRDC. Normally the GRDC would expect income from a season producing about 20 million tonnes of wheat and 10 million tonnes of barley. Receivals of grain are looking like being less than 10 million tonnes of wheat and 4 million tonnes of barley. The GRDC budgets have been scrutinised and both administration and R&D budgets for 06/07 and 07/08 have been reduced. The reduction has been dampened by the use of accumulated reserves by the GRDC. The GRDC board approved using more reserves this year than had previously been agreed. These budgets are based on a normal 07/08-year of million tonnes of wheat and 10 million tonnes of barley. If this is not achieved then more reductions to R&D expenditure will have to be expected. In terms of the New Grain Products portfolio this R&D reduction has translated into prospective contracting of three very high priority projects for 07/08 out of ten. Aquaculture projects are more at the discovery end of the portfolio for New Grain Products and would not have ranked highly enough to be funded this year.

GRDC Feed grain in general

GRDC supports R&D in a number of different feed areas. One program that has been supported for a number of years is the Premium Grains for Livestock Program (PGLP). This program has found that different grains vary in the amount of energy they can give to particular animals and this digestible energy can be measured by NIR analysis. The GRDC has contributed $1.9 million over 5 yrs, which is 45% of the total contribution for this program.

The Varieties Line of Business (LOB) has a number of projects to do with feed grains. This LOB orchestrated the reorganisation of the Australian Barley breeding into 3 nodes. The northern node concentrates on feed and defaults to malt, while the other two nodes breed for malt and default to feed. The Varieties LOB is committed to two Triticale breeding programs. The University of Sydney program is focused on improving Triticale production through breeding and agronomy to develop Triticale as a profitable feed grain. The other program is contained in AGT.

New Grain Products is working with the Livestock and Feed Grain User Group (LFGUG) in regards to participating in the “Feed grain Partnership”. The idea of the partnership is to create a forum to discuss projects in the feed grain area that will benefit from information sharing and input from the whole of the feed grain supply chain. New Grain Products also supports two projects in the Pork CRC called “Enhancement of NIR calibrations for predicting the energy value of weather-damaged grains for pigs” and “Selection of sorghum grains for enhanced nutritional value for pigs of residues remaining from ethanol production”.

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