HARVESTING THE BENEFITS OF LUPIN MEALS IN AQUACULTURE FEEDS

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ABSTRACT

In order to reduce risk associated with being too dependent on fish meal and fish oil use in aquaculture feeds, considerable effort has been expended both in Australia and internationally to assess and develop a range of grain protein meals as alternatives. From early initiatives in the 1990s lupins emerged as one the grain sources with potential for further development. To progress this opportunity the Aquaculture Feed Grains Program (AFGP) (an initiative of the Grains Research and Development Corporation) was created which incorporated a broad multidisciplinary team working with a range of aquaculture species and grain products to facilitate the commercialisation of lupin use in fish feeds. However, the development process underpinning the acceptance and adoption of the use of the grain resource has been a long and detailed process and rests heavily on the technical assessment of this grain.

The AFGP initiative commenced with a broad assessment of a range of grain raw materials. During this initial assessment phase lupins emerged as one of the grains with exciting potential as an aquaculture feed ingredient. Digestibility studies were used to intimately screen raw materials, and were later also used to refine the quality assessment (QA) process. The feeding studies used to undertake the digestibility work also allowed for assessment of feed intake variability as a consequence of the different samples being tested. Over a five-year period over 120 lupin kernel meal samples were assessed for their digestibility parameters. This sample and data set has since been used to create the world's first viable NIRS calibration for digestible protein and energy from a grain protein ingredient for use in fish diets.

To consolidate the digestibility and palatability data a series of growth were also undertaken. The growth studies were used to examine a range of features associated with lupin use including the influence of alkaloids, the implications of variability in digestible nutrient and energy values and the efficiency of grain protein utilisation among others. Consistently lupins have proven to be highly nutritious protein and energy sources that have few nutritional problems with high (> 20%) inclusion levels in fish diets and in some cases even improved the performance of the diets relative to fishmeal controls and soybean meal based diets. Further work was also undertaken to examine the processing implications of using lupins in modern extruded fish feeds. Using a laboratory-scale twin-screw extruder a wide range of feeds was produced and the physical qualities of the pellets subjected to a range of tests. From this work it was shown that lupins also have some unique functional properties to contribute to aquafeed pellets.

The Australian commercial extension process was aided by the direct involvement of commercial grain processors and feed producers as part of the program. Uptake of the research by these sectors underpinned the development of the export of Australian lupins to aquafeed markets throughout the world. To further assist the technology extension process internationally, market technical tours were conducted in 2004, 2005 and 2006 to Japan, Thailand, Norway, Denmark, Scotland and Chile.

From this research a major initiative was progressed in Western Australia with the development of the world's largest commercial lupin dehulling facility. This AUD$8million facility was developed with the aquaculture feed market identified as one its key markets. The future of lupins as a key raw material for use in aquaculture feeds remains promising.

KEYWORDS

aquaculture, grain, lupin, feed, quality

INTRODUCTION

There is substantial risk associated with being too dependent on fish meal and fish oil use for aquaculture feeds. To address this risk considerable effort has been expended both in Australia and internationally to assess and develop a range of alternative raw materials. During the 1990s the Fisheries Research and Development Corporation (FRDC) initiated the assessment of a broad range of raw materials when fed to Atlantic salmon, Prawns, Barramundi and Silver perch (Allan *et al.* 1998a; 1998b; Booth *et al.* 2004). From these early initiatives lupins emerged as one the grain raw material options with potential for further development.

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

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

Table 2. Variability in lupin kernel meal composition (% dry matter) (n = 75).
Derived from Glencross *et al.* (2008a).

	Mean	SD	CV%	Min.	Max.
Dry matter	91.6	0.6	0.6	90.4	92.8
Crude protein (N x 6.25)	45.4	3.4	7.6	36.5	56.7
Fat	7.8	0.9	12.1	5.2	9.7
Ash	3.0	0.4	14.0	1.9	3.9
Carbohydrate	43.8	3.3	7.6	32.7	53.9
Phosphorus	0.4	0.1	15.3	0.3	0.6
Energy	20.8	0.3	1.5	20.1	21.5
Sum of amino acids	44.0	3.2	7.2	33.2	53.7
Alanine	1.6	0.1	6.8	1.3	1.8
Arginine	5.1	0.5	9.9	4.0	6.6
Asparagine	4.9	0.4	7.7	3.8	5.9
Cysteine	0.7	0.1	16.5	0.5	1.3
Glutamate	10.0	0.8	7.8	7.5	12.6
Glycine	1.9	0.1	6.4	1.5	2.1
Histidine	1.1	0.1	11.8	0.8	1.4
Isoleucine	1.7	0.1	7.6	1.3	2.0
Leucine	3.2	0.3	8.0	2.4	4.3
Lysine	1.8	0.2	13.2	1.2	2.4
Methionine	0.3	0.1	32.2	0.2	0.7
Phenylalanine	1.8	0.2	12.4	0.1	2.1
Proline	2.5	0.6	26.0	1.0	4.3
Serine	2.4	0.2	6.8	1.9	2.9
Threonine	1.8	0.1	7.3	1.5	2.1
Tyrosine	1.7	0.2	9.1	1.1	2.1
Valine	1.5	0.1	8.4	1.2	1.8
Crude fibre	30.9	4.6	14.9	17.5	43.4
Neutral-detergent fibre	10.2	5.4	52.3	5.2	26.2
Acid-detergent fibre	6.6	4.5	69.1	3.0	20.0
Lignin	0.7	0.5	65.9	0.2	2.2

CV%: Coefficient of variation = SD/Mean x 100.

Initial efforts to develop new markets for lupins focused on Southeast Asian fish species and had limited success. In 2000 a review was undertaken of the strategy to target Southeast Asian aquaculture feeds and the review suggested that the more advanced aquaculture feed sectors, such as Atlantic 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. Therefore by focusing development attention on the international salmon feed sector it was also recognised that the other aquaculture sectors would be likely to take on the technology once it had gained acceptance in that sector.

To progress this opportunity the Aquaculture Feed Grains Program (AFGP) (an initiative of the Grains Research and Development Corporation (GRDC) and the Centre for Legumes in Mediterranean Agriculture (CLIMA)) was created in 2002 to incorporate a broad multidisciplinary team assembled to work with a range of aquaculture species and grain products. The main aim of this program was to facilitate the commercialisation of lupin use in fish feeds.

A key thrust of the program in product screening and development work was conducted with rainbow trout as a "lab-rat" species, with results that were later cross-referenced with other species, showing that it is highly a suitable model, particularly for Atlantic salmon. To further evaluate the grain products identified as priorities, two-key additional aquaculture species of Atlantic salmon and prawns were used.

GRAIN VARIABILITY

There is considerable variability in the composition of lupin meals depending on both lupin species and whether they are in a whole-seed or processed form and again what sort of processing has been used (Table 1). The 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* species, which both exceed 50% protein in the kernel meal form. Lipid content is highest in the *L. mutabilis* and *L. albus* varieties. Lupins are notable in that they possess negligible starch levels within their carbohydrate component.

Within the different lupin species significant variability in composition has been observed (Glencross *et al.* 2008a) (Table 2). There are both genetic and environmental factors that influence this composition variability. 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.

DIGESTIBILITY STUDIES WITH LUPINS

The effect of dehulling efficiency on protein and energy digestibility has been clearly demonstrated (Fig. 1). A single-source batch of *L.* angustifolius seed (cv. Coromup), 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%). These findings were reinforced by subsequent work using refined methodologies (Glencross *et al.* 2005). The digestibility of lupin kernel meal to Tiger shrimp (*Penaeus monodon*) was also clearly greater than that of whole seed meal. Recent studies on several cultivars of *L. angustifolius*, have shown that the protein digestibility of the kernel meals were uniformly high with an average of 94% (Smith *et al.* 2007a). 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.

The variability in the digestibility of protein and energy of different cultivars of *L. angustifolius* kernel meal when fed to rainbow trout was studied by Glencross *et al.* (2008b) who found significant variability among different samples irrespective of genotype or crude composition (Fig. 2). Expansion of this data-set (n = 120 lupin samples), has produced the largest single grain type evaluation ever undertaken for an aquaculture species. So far from this work it has been found that there was substantial variability in digestibility of both protein and energy from lupin kernel meals and that this variability correlates well with crude protein and lignin content of the meals (Fig. 3; Glencross *et al.* 2008a).

Table 3. 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	-	-

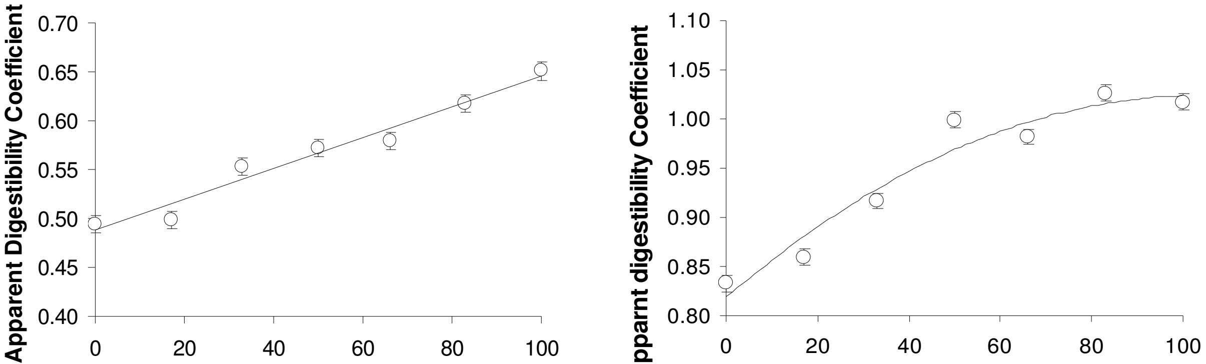

Fig. 1. Digestibility of energy (A) and protein (B) from lupins with increasing dehulling efficiency. Derived from Glencross *et al.* (2007b).

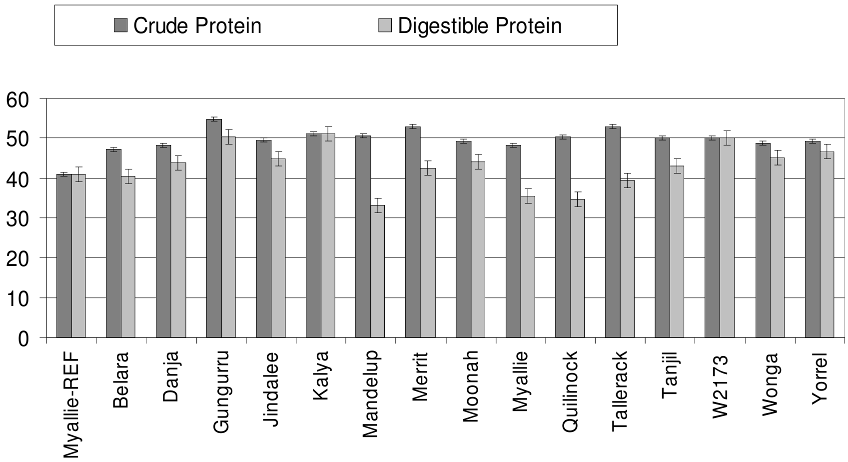

Fig. 2. Digestibility of protein and energy from lupin kernel meals according to *L. angustifolius* cultivar type. Notable is the significant variability among the different cultivars and even within cultivars from different years and locations. Derived from Glencross *et al.* (2008b).

The data set was further explored to enable the development of a viable calibration set for near infra-red spectroscopy (NIRS).

Digestibility assessment was also used in the development and assessment of protein concentrates developed from lupins (Fig. 4). A series of protein concentrates and isolates produced from lupins with increasing protein concentration showed 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; 2005)

GROWTH STUDIES WITH LUPINS

To consolidate the digestibility and palatability data a series of growth studies have also undertaken. The growth studies have examined a range of issues associated with lupin use including the effect of serial inclusion of different lupin varieties, the implications of variability in digestible nutrient and energy values, the efficiency of grain protein utilisation and the influence of alkaloids, among others. Consistently lupins have proven to be highly nutritious protein and energy sources that have few nutritional problems with high (> 20%) inclusion levels in fish diets and in some cases even improved the performance of the diets relative to fishmeal controls and soybean meal based diets.

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% (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 (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. Notably, feed intake was not influenced by *L. luteus* kernel meal inclusion level.

There are few comparative studies comparing the inclusion of different lupin species with soybean meal in diets fed to any species. Smith *et al.* (2007b) compared two different varieties of *L. angustifolius* kernel meal and soybean meal when fed to tiger shrimp (Penaeus monodon) and found no negative effects with inclusion of lupin meals at up to 43% inclusion. A study by Glencross *et al.* (unpublished) with rainbow trout compared *L. angustifolius*, *L. luteus* kernel meals and a soybean meal included at 0%, 10%, 20%, 30% and 40% inclusion. No negative effects of inclusion of either lupin at any inclusion level were observed, though a reduction in growth performance was observed with the 40% inclusion of soybean meal (Fig. 5).

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 (Glencross *et al.* 2008c). 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 supported the hypothesis that this grain protein source is as an effective energy and protein source as fishmeal when considered on an equivalent digestible basis. This proves that fish can utilise plant proteins as effectively as they can use fish based proteins.

Although lupin kernel meals and protein concentrates have been shown to be able to be included in diets up to 40% (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 of any one alternative raw material. Typically more realistic commercial inclusion levels for alternative raw materials in practical feeds are of the order of 10% to 20% depending on price and protein content.

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). Gramine was examined because it was noted to be the primary alkaloid variety in *L. luteus*, and critical thresholds for gramine were required to be known for refinements in the grain's breeding program. The critical level for feed intake reduction and reduced growth when included in diets fed to rainbow trout was between 100-500 mg/kg diet.

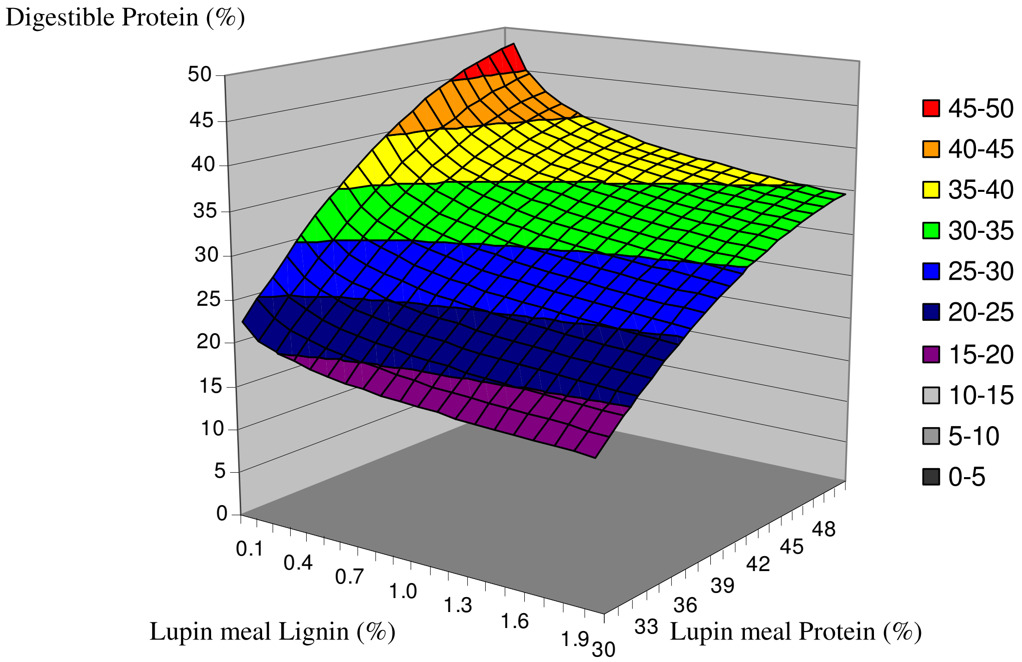

Fig. 3. Model of the dual influence of lupin meal protein (%DM) and lignin (%DM) on the digestible protein content (%DM) of lupin meal when fed to rainbow trout. Derived from Glencross *et al.* (2008a).

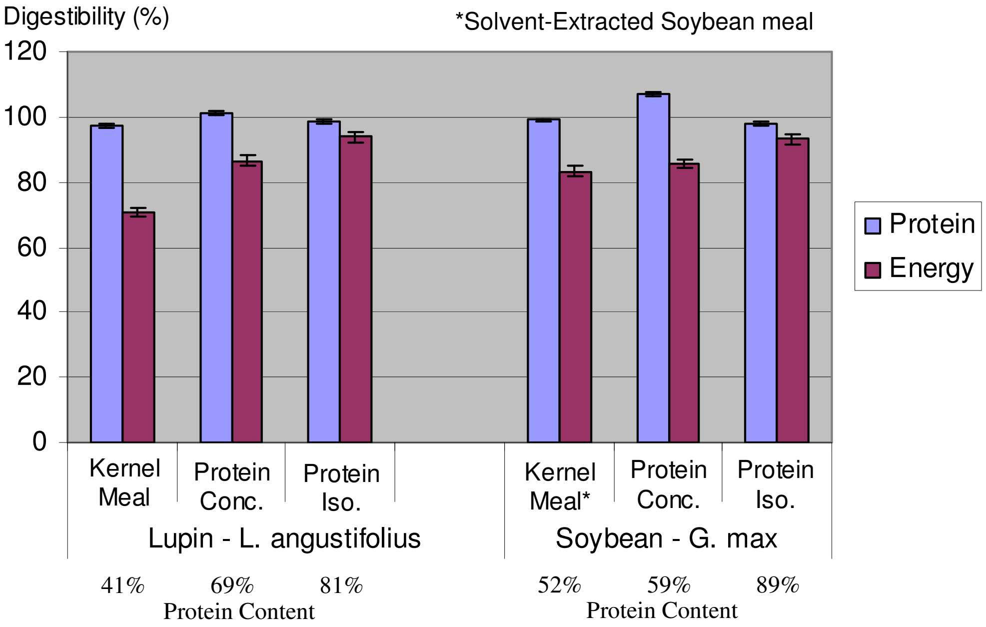

Fig. 4. Model of the dual influence of lupin meal protein (%DM) and lignin (%DM) on the digestible protein content (%DM) of lupin meal when fed to rainbow trout. Derived from Glencross *et al.* (2008a).

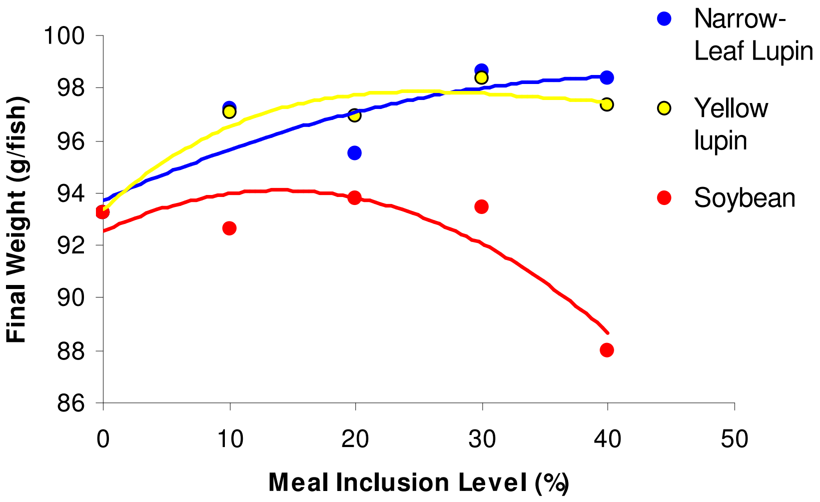

Fig. 5. Growth of rainbow trout fed diets with increasing levels of *L. angustifolius* cv Myallie kernel meal, *L. luteus* cv Wodjil kernel meal and solvent extracted soybean meal. From Glencross *et al.* (unpublished).

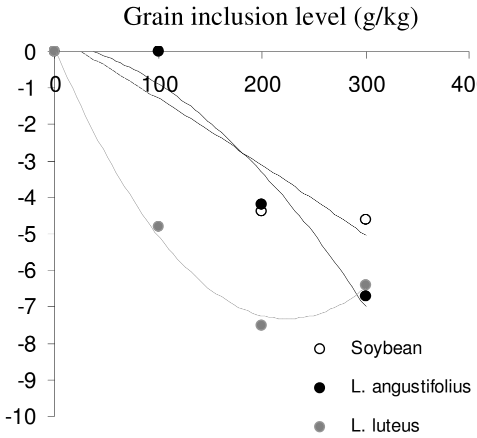

Fig. 6. Pellet sink rates (cm/s) as a function of grain meal inclusion.

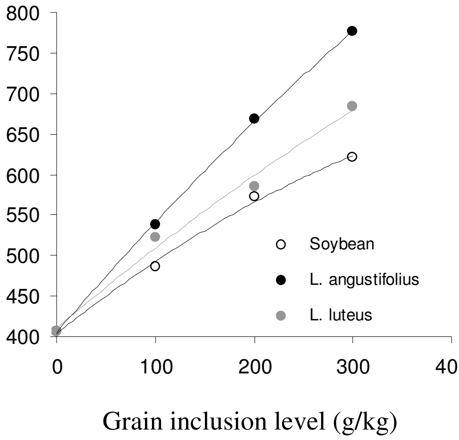

Fig. 7. Pellet hardness (g of force to split pellet) as a function of grain meal inclusion.

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 was examined in diets fed to rainbow trout using a cross-referencing method of both chemical and enzymatic removal of oligosaccharides (Glencross *et al.,* 2003b). 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 and unorthodox diet formulations, 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.

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. The intestinal histology of salmon fed a range of diets, including soybean and *L. angustifolius* and *L. luteus* kernel meals resulted in a significantly lower level of intestinal damage being observed from the two lupin treatments compared to the soybean meal. *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 is reputed to be caused by the saponin content of soybeans. 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.

FEED PROCESSING

Further work was also undertaken to examine the processing implications of using lupins in modern extruded fish feeds. This aspect is usually referred to as the functional or technical qualities of a raw material (Thomas and van der Poel, 2001). Using a laboratory-scale twin-screw extruder a wide range of feeds was produced and the physical qualities of the pellets subjected to a range of tests. From this work it was shown that lupins also have some unique functional properties to contribute to aquafeed pellets.

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 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 (Fig. 5). However the effect of *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 (Fig. 6). 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.

INDUSTRY ADOPTION

In Australia the commercial extension process was aided by the direct involvement of commercial grain processors and feed producers as part of the program. Uptake of the research by these sectors underpinned the development of the export of Australian lupins to aquafeed markets throughout the world.

Since the initiation of the program, several major industry initiatives have taken place. 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 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, Japan, 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 technology in the form of NIRS for rapid analysis of crude composition and digestible protein and energy values. Each of the samples evaluated were also 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.

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 was 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 (cv. 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 *L. angustifolius* variety cv. Coromup was released. This variety is one of the first higher-protein varieties to be released and reverses a trend of decreasing protein in Australian *L. angustifolius* varieties that has been occurring over the past 10 years.

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 3). 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.

FUTURE HORIZONS

The future of lupins as a key raw material for use in aquaculture feeds remains promising, provided there is continued production of lupins as a feed grain and that efforts are maintained towards maintaining and/or improving the principle quality criteria by which this feed grain is valued. Further directed studies to understand the chemical and physical components that affect raw material digestibility will provide an improved capacity to predict nutritional value prior to application of the raw material to feeds.

Further work is also required with additional target market species to improve the application of this feed grain to other species such as tilapia and catfish. This should also support an improved capacity to export lupins and lupin products to Southeast Asian countries.

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