Feeding lupins to fish : A review of the nutritional and biological value of lupins in aquaculture feeds

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Contents

Common abbreviations used

ABV	Apparent Biological Value
ADC	Apparent Digestibility Coefficient
ADN / E / P	Apparent Digestible Nitrogen / Energy / Phosphorus
AF	As Fed
d	Day
CF	Crude Fat
CP	Crude Protein
cv.	Cultivar
DE	Digestible Energy
DGC	Daily Growth Coefficient
DM	Dry Matter
ERV	Energy Retention Value
FCR	Food Conversion Ratio
g	Gram
kg	Kilogram
mg	Milligram
LYS	Lysine
MET	Methionine
MJ	MegaJoule
NFE	Nitrogen Free Extractives
NSP	Non Starch Polysaccharide
PER	Protein Efficiency Ratio
PPV	Productive Protein Value

Definitions of standard terms used

Apparent Biological Value (ABV)

The proportion of digestible nutrient intake that is retained as growth. This parameter is a derivation of nitrogen retention and nitrogen digestibility.

Apparent Digestibility Coefficient (ADC)

A coefficient used to describe the amount of food absorbed by an animal when fed a specific diet or ingredient. Usually presented on a percentage basis. Determined by comparison of relative concentration between feed and faeces of an indigestible component (marker) of a given diet. Is calculated as:

$$ADC = 100 - 100 \times \left(\frac{\text{marker content of feed}}{\text{marker content of faeces}} \right)$$

Apparent Digestibile Nitrogen / Energy / Phosphorus (ADN / E / P)

A coefficient used to describe the amount of a specific nutrient (usually nitrogen/protein, energy or phosphorus) absorbed by an animal when fed a specific diet or ingredient. Usually presented on a percentage basis. Determined by comparison of relative concentration between feed and faeces of an indigestible component (marker) and nutrients of a given diet. Is calculated as:

$$\text{ADN / E / P} = 100 - 100 \times \left(\frac{\text{marker content of feed}}{\text{marker content of faeces}} \times \frac{\text{nutrient content of faeces}}{\text{nutrient content of feed}} \right)$$

As Fed (AF)

That content of a substance as provided in its usual state, i.e. not corrected for moisture content.

Daily Growth Coefficient (DGC)

A growth coefficient based on the one-third exponent of an animals weight gain. For specific validation and comparison of measures of growth in fishes see; Kaushik (1998a). DGC is calculated as:

$$\text{DGC} = \left(\frac{W_t^{1/3} - W_i^{1/3}}{t} \right) \times 100$$

*Where $\boldsymbol{W_t}$ represents the animal's weight at time period **t**, and $\boldsymbol{W_i}$ represents the animal's weight at the beginning of the time period.*

Dry Matter (DM)

That content of a substance when the substance is devoid of water.

Energy Retention Value (ERV)

See Nitrogen/Energy/Phosphorus Retention.

Food Conversion Ratio (FCR)

The amount of food consumed to produce one kilogram of live-weight gain of the animal. Usually this value is determined on an As Fed basis of the feed to live-weight gain, but in some circumstances a Dry Matter value of the feed is used (FCR_{DM}).

Nitrogen Free Extractives (NFE)

The content of a diet, minus the water, protein, fat and ash contents. Represents a crude assessment of the carbohydrate content of a substance.

Nitrogen (Energy / Phosphorus) Retention

The amount of nitrogen (or energy or phosphorus) retained by an animal when fed a specific diet. This parameter provides an indication of the potential a diet has to support biosynthetic activity. Also often referred to as PPV (Productive Protein Value) or ERV (Energy Retention Value). Calculated as:

$$\text{Nitrogen (Energy / Phosphorus) Retention} = \left(\frac{\text{Nt} - \text{Ni}}{\text{Nc}} \right) \times 100$$

*Where **Nt** is the nitrogen content of the animal at time **t** and **Ni** is the initial nitrogen content of the animal. **Nc** is the amount of nitrogen consumed by the animal from initial assessment to time **t**. Determination of Energy and Phosphorus retention is achieved the same way, but with the substitution of the relevant energy and phosphorus criteria where the corresponding nitrogen criteria are indicated in the equation. Typically, this figure is determined based on gross nitrogen / energy / phosphorus intake, though more accurate assessments will determine it based on digestible intake (see Apparent Biological Value). Notably this figure will be influenced by animal size, therefore it is important that comparisons are made only between animals of similar initial size.*

Protein Efficiency Ratio (PER)

The amount of live-weight gain per protein intake. This parameter is derived from FCR and protein content of the feed fed to the animal to achieve that FCR.

iii. Summary

iii.i. Generic nutritional value

In all aquaculture species for which a nutritional assessment has been made on the value of lupins, they have been shown to be a well-accepted and nutritionally useful ingredient. The extent of this value varies between species and also between studies.

iii.i.i. Dry matter utilisation

The amount of solid waste output from fish fed lupin meals is consistent with that observed of most other plant protein resources. The dry matter digestibilities of lupin meals are strongly influenced by the high levels of non-starch polysaccharides (NSP) within the grain (Morales et al., 1994; Gomes et al., 1995; McMeniman, 1998; Allan et al., 1998a; Burel et al., 2000a; Smith et al., 2000). The high reliance on protein metabolism in most aquaculture species and a lack of capacity by most fish species to deal with dietary NSP means that essentially the entire NSP component of lupins is defaecated. At high levels, it is also likely that NSP may act as an anti-nutrient, effectively acting like fibre and inhibiting the digestive process for other nutritionally valuable components of the feed. Accordingly, substantial improvements are made in the level of dry matter digestibility with the removal of the lupin seed coat, which is concomitant with a reduction in the levels of NSP within the meals (Allan et al., 1998a; Smith et al., 2000).

iii.i.ii. Nitrogen/Protein utilisation

High levels of nitrogen/protein digestion have been reported from essentially all species in which lupins have been evaluated (Morales et al., 1994; Gomes et al., 1995; McMeniman 1998; Allan et al., 1999a; Burel et al., 2000a; Smith et al., 2000). In many instances the digestibility of lupin protein has been significantly superior to that of many other plant protein and/or animal protein resources (Hughes, 1988; Gomes et al., 1995; McMeniman 1998; Burel et al., 2000a; Smith et al., 2000; Booth et al., 2001). Little difference in protein digestibility has been reported between the key lupin species. Notably though, the lupin kernel meals have protein digestibilities substantially greater than that of the whole-seed meals in most species studied (Robaina et al., 1995; McMeniman 1998; Allan et al., 1998a). As with many other protein resources, cooking or autoclaving of lupin meals reduces the nutritional value of their protein content (De la Higuera et al., 1988; Vandepeer et al., 1999).

Figure 1. Lupin kernels, kernel meal and seed

Table i.i *Summary of the nutritive value (Digestibility%) and Available nutrient content of lupin whole-seed and kernel meals and defatted soybean meals in various aquaculture species as determined on an ingredient specific basis*

	Digestibilities (%)				Available Nutrients		
Species and Ingredient	Dry Matter	Nitrogen	Energy	Phosphorus	Protein (g/kg)	Energy (MJ/kg)	Phosphorus (g/kg)
Rainbow trout (*Oncorhynchus mykiss*)							
L. angustofolius (whole seed meal)	-	85.5	61.2	-	275	11.1	-
L. albus (kernel meal)	69.7	96.2	77.0	61.9	385	15.7	2.2
Defatted soyabean meal	71.2	90.1	56.0	22.0	439	9.9	1.5
Silver Perch (*Bidyanus bidyanus*)							
L. angustofolius (whole seed meal)	57.0	91.8	51.0	-	296	9.2	-
L. angustofolius (kernel meal)	68.0	93.3	61.9	-	364	11.7	-
L. albus (whole seed meal)	64.7	96.1	72.7	77.5	344	14.2	2.8
L. albus (kernel meal)	77.8	101.4	85.2	73.8	406	17.4	3.7
Defatted soyabean meal	73.0	95.0	82.0	-	463	14.4	-
Tiger Prawn (*Penaeus monodon*)							
L. angustofolius (whole seed meal)	67.0	94.0	68.0	-	303	12.3	-
Defatted soyabean meal	67.0	92.0	71.0	-	448	12.5	-
Greenlip Abalone (*Haliotis laevigata*)							
L. angustofolius (whole seed meal)	-	91.0	80.0	-	293	14.5	-
L. luteus (whole-seed meal)	61.0	91.0	83.0	84.0	349	16.3	3.6
Defatted soyabean meal	57.0	87.0	84.0	86.0	424	14.8	6.0

Indicated values within species are not necessarily derived from the same study. See main text of review for specific details and appropriate references.

iii.i.iii. Energy utilisation

The highly digestible protein and lipid components of lupin meals constitute almost the entire digestible energy value of this grain resource (Allan et al., 1998a; Burel et al., 2000a; Kissil and Lupatsch, 2000). Accordingly, slight differences in the energetic value have been identified between *L. albus* and *L. angustifolius*, primarily in response to the higher protein and fat levels in *L. albus*. Calculated assumptions of energy utilisation from protein and energy digestibilities support that little digestible energy contribution of the NFE component is occurring when aquaculture species are fed any of the lupin grain commodities (Morales et al., 1994). Similar to the dry matter digestibility characteristics, substantial improvements are made in the level of energy digestibility with the removal of the lupin seed coat (Allan et al., 1998a; Smith et al., 2000).

iii.i.iv. Phosphorus utilisation

The assessment of phosphorus utilisation is increasingly becoming a more important nutritional parameter in accordance with an increasing imperative towards environmental best-management practice from aquaculture. From the data available on phosphorus utilisation, the digestibility of phosphorus from lupins is considerably better than that seen for essentially any other protein resource (Allan et al., 1998a; Burel et al., 2000a). Phosphorus digestibilities almost twice those reported in the same species fed many other plant protein meals have been reported (Carter and Hauler 1999).

iii.ii. Generic biological value

Assessment of the biological value of a range of species and processing forms of lupins, across a range of aquaculture species, also clearly shows a good capacity by fish and crustaceans to use this ingredient in compound feeds.

iii.ii.i. Inclusion levels

There is considerable variability in the maximum reported inclusion level of lupin meals in diets for aquaculture species with values ranging from 20% to 70% (De la Higuera et al., 1988; Robaina et al., 1995; Burel et al., 1998; Williams, 1998; Sarac et al., 1998). As with other protein resources, the maximum inclusion is likely to be a function of the protein content of resource used, the protein requirements of the animal and the level of feed attractants and ingestants included in the diet. On this basis, *L. luteus* has potentially more value than both *L. albus* and *L. angustifolius*. Regardless of lupin species though, their kernel meals have considerably more potential than their respective whole-seed meals for most species. Any restrictions on the use of high levels of inclusion of any lupin product can easily be circumvented by blending with other protein resources or the use of crystalline methionine (Rodehutschord et al., 2000). However, in practical terms, as with most other plant protein resources, the limitation on the inclusion level is more likely to be one based on level of risk aversion, faecal waste output and diet processing characteristics.

iii.ii.ii. Nitrogen retention

The level of nitrogen retention that has been observed from lupins fed to a range of aquaculture species also provides good evidence for the nutritional quality of this protein resource (Robaina et al., 1995; Burel et al., 1998; Carter and Hauler, 1999). Generally, nitrogen retention was equal to that observed from the range of other ingredients examined. For most species, the level of retention in iso-nitrogenous diets was improved relative to control diets with moderate inclusion of lupin meals in the diet, though with higher inclusion level the nitrogen retention often deteriorated. Based on the work of Williams (1998), this deterioration may be consistent with the selective catabolism by these species of the relatively high levels of non-essential amino acids present in diets with high levels of lupin meals.

iii.ii.iii. Energy retention

The level of energy retention observed from lupins fed to a range of aquaculture species is highly consistent of the level of crude protein in the grain (Kissil and Lupatsch, 2000). While it is presumed that some benefit is derived from the fat content of lupins, the low levels of this nutrient (56 to 114 g/kg) in the range of lupin resources available mean that in most cases it is unlikely to be the key parameter in influencing the energetic value of lupins to an aquaculture species. In essentially all species, the carbohydrate content of lupins has been observed to have negligible digestible energetic value (Morales et al., 1994). Notably, lupins contain negligible starch content.

iii.ii.iv. Phosphorus retention

Similar to observations of nitrogen retention, phosphorus retention by aquaculture species fed lupins was also improved with the inclusion of lupins, though unlike nitrogen retention, as inclusion levels of lupin resources increased beyond moderate inclusion levels, so to did the level of phosphorus retention (Burel et al., 1998; Burel et al., 2000b). This aspect of lupin use has considerable implications for the use of lupins in the diets of fin-fish in regard to reduction of phosphorus output of aquaculture (Ballestrazzi et al., 1994; 1998; Bedford, 1996; Ervik et al., 1997; Medale et al., 1998). Coupled with the already low levels of phosphorus in lupin meals (3.0 to 9.7 g/kg), the relatively high levels of phosphorus digestibility and the high levels of phosphorus retention mean that the total soluble outputs of phosphorus by fish fed lupins will be substantially lower than that of fish fed other plant ingredients, and particularly other animal derived ingredients.

Figure 2. Shrimp and lupins

iii.iii. Generic processing value

Processing of diets, for fin-fish, that contain lupins has shown that lupin meals can be easily used as an ingredient in diets made using extrusion technology. Resulting diets have a range of characteristics, depending on the processing conditions used. Diets extruded with a lupin component tend to have less expansion and oil absorption than that achieved with some other plant meals, though they have higher bulk density and faster sink rates. Notably the pellets are also usually stronger and more durable (Gleeson et al., 1998a).

Diets made using pellet press technology have shown relatively poor pellet stability after extended immersion in water. Considerable differences were found between the processing form of the lupins (ie. whole-seed meal, kernel meal or protein concentrate). However, it is likely that the effects of the processing form on pellet stability are also related to protein content and consequently the amount of wheat flour filler that has been used in the relevant diets (Sudaryono et al., 1999a; 1999b)

Figure 3. Red seabream with lupin kernels, lupin kernel meal and lupin (L. angustifolius cv. Gungarru) seeds

1.1 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 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 (Gladstones, 1998; Perry et al., 1998).

The three key commercial species of lupins are *L. angustifolius* (Narrow-leafed Sweet Lupin), *L. albus* (White or Albus Lupin) and *L. luteus* (Yellow Lupin). *L. angustifolius* dominates world lupin production, with the bulk of the grain (77% of world production), being produced in the mediterranean climate of south-western Australia (Perry et al., 1998). The primary cultivars of *L. angustifolius* grown are the Gungurru and Merrit varieties. Recent development has seen the release of the Myallie, Belara and Tallerack varieties, which were developed to suit different environmental and production criteria (GPWA, 2000). Both *L. albus* and *L. luteus* are also grown in this region and in several other regions in Australia, but at much lower quantities (Perry et al., 1998; Petterson et al., 1998).

Production of lupins in other countries focuses primarily on *L. albus*, with significant tonnages being produced in Chile, Egypt, South Africa and Eastern Europe (primarily former USSR, Germany and Poland) (Perry et al., 1998) and *L. luteus* in Poland. Other species with commercial potential include *L. atlanticus*, *L. cosentini* and *L. mutabilus*. None of these species are cultivated in large quantities and their practical value as a feed resource is not considered overly viable at present (Perry et al., 1998).

Lupin grain has been used as a key feed ingredient in diet formulations for terrestrial species; indeed this is the primary use of the grain (Gdala et al., 1996; Edwards and van Barneveld, 1998; Petterson, 2000). The capacity to use lupins as either a whole-feed or feed ingredient have been well studied in most terrestrial domestic animal species, as have the nutritional requirements and the physiological and biochemical processes associated with nutrition in these species (reviewed in: Edwards and van Barneveld, 1998; van Barneveld, 1999 and Petterson, 2000).

Figure 5. L. angustifolius (cv. Gungarru) seed with kernels

1.2 Lupins as a feed ingredient

In comparison to most terrestrial species, the nutritional knowledge of aquaculture species, including their nutritional requirements, the associated physiological and biochemical processes, and the capacity of various ingredients to be used in these processes, is limited (Edwards and van Barneveld, 1998). A notable difference, however, that has been identified in many fin-fish species in particular, is a relatively higher dietary protein requirement than for terrestrial domestic species (NRC, 1993). This high need for dietary protein is to satisfy two primary nutritional needs, amino acids and energy.

As a consequence of the limited capacity of most aquaculture species to utilise dietary carbohydrates for energy (NRC, 1993), aquaculture nutritionists are usually forced to rely on the use of either dietary fat or protein to satisfy energetic requirements of the fish being fed. Accordingly, dietary protein is usually over specified on an amino acid requirement basis in order to maximise the dietary energy content. As a consequence of this oversupply of dietary protein, the importance of the amino acid profile of the protein is less important than that required in diets for terrestrial monogastric species, such as pigs and poultry (Edwards and van Barneveld, 1998).

To date, this need for protein has been provided in most fin-fish and crustacean diets through the inclusion of fish meal in the diet. However, with the continuing expansion of the aquaculture industry the need for alternative protein resources to fishmeal is an increasing imperative (New and Csavas, 1993; Tacon, 1996). To date there has been considerable research to examine the use of plant-based alternative protein resources (Arnesen et al., 1989; Gomes et al., 1995; Booth et al., 2000). Soybean meal has been widely used, with considerable success (Arnesen et al., 1989; Medale et al., 1998; Refstie et al., 1998; Storebakken et al., 1998b; Vielma et al., 2000). Comparison of the composition of lupin and soybean meals suggests that there could also be considerable potential for the use of lupin meal in aquaculture diets. Several studies have confirmed this potential (see Section 4 in this review). Notably, it has been pointed out by van Barneveld (1999) that feed formulators and nutritionists are seldom looking for the “perfect” ingredient from which to make diets, but rather a suite of complementary ingredients of consistent and reliable quality that can be blended together to provide nutritionally complete diets. In this sense, while lupins or any other plant protein resource are not an ideal complete feed, they do provide an option as a highly consistent, nutritionally valuable ingredient.

This review examines several facets of the use of lupins in diets for aquaculture species. First, a comprehensive examination of the composition of lupins is presented, detailing the physical chemistry of this grain and the variations that occur between species and processing forms. Second, is a review of the work published to date where lupins have been fed, either as an ingredient in a compound feed, or as a whole-feed, to an aquaculture species. Third, is a review of the influences of lupins on feed processing characteristics that have been identified in the aquaculture feeds sector.

2.1 Lupin composition

2.2 Protein and amino acids

2.2.1 Crude protein

Lupin seeds are typified by a higher protein content (310 to 420 g/kg DM) than most other grain legumes (pulses). There is considerable variation in the protein content between the various species and between cultivars and even within cultivars as a result of the characteristics of the growing season and soil type (Petterson et al., 1997).

Yellow lupin (*L. luteus*) is generally regarded as having the highest protein content of the lupin species, with whole seeds typically having protein levels of 400 to 450 g/kg DM. The seed kernel typically has a protein content around 530 g/kg DM, though this has been reported to exceed 570 g/kg DM (Petterson et al., 1997, Table 2.1). Albus/White lupins (*L. albus*) are also high in protein with the whole seed having protein levels of 320 to 440 g/kg DM and the kernel yielding about 460 g/kg DM. Albus lupins also tend to have a thinner seed coat and as a consequence removing the seed coat of this variety does not increase the protein content of the resulting kernel meal to the same degree as is observed in Yellow and Australian Sweet varieties (Evans, 1998). Australian sweet lupins (*L. angustifolius*) are the predominant variety produced commercially in Australia, and form an important ingredient in the rations of many pig and poultry formulations. *L. angustifolius* has a whole seed protein content of about 300 to 410 g/kg DM, with a kernel protein content of about 440 g/kg DM, though this typically closer to 420 g/kg DM under industrial dehulling conditions. Some specimens have had protein levels of up to 540 g/kg DM (Petterson et al., 1997).

Figure 6. Seeds, kernels and meals of (L to R) Soybeans, L. angustifolius, L. luteus and L. albus,

Table 2.1 Chemical composition (g/kg) of lupin seed and kernel meals and soybean meal on an as received basis

Nutrient	**Whole seed meal**			**Kernel meal**			Solvent-extract
	L. angustifolius	*L. albus*	*L. luteus*	*L. angustifolius*	*L. albus*	*L. luteus*	Soybean meal
Dry matter	911	914	915	900	905	917	890
Crude protein	322	358	383	390	400	525	487
Crude fat	58	95	56	70	114	72	11
Nitrogen Free Extract	504	428	441	408	358	277	329
Total calcium	2.2	2.0	2.2	1.0	-	1.2	4.0
Total phosphorus	3.0	3.6	4.3	5.1	5.0	5.5	7.0
Crude fibre	149	103	162	87	18	17	-
Acid detergent fibre	197	143	249	70	-	31	110
Neutral detergent fibre	227	172	343	71	-	48	130
Ash	27	33	35	27	33	43	68
Gross energy (MJ)	18.1	19.6	19.6	18.9	20.4	20.0	17.6

Values based on data from “The chemical composition and nutritive value of Australian pulses”. GRDC Final Report. Petterso et al. (1997), GPWA (2000), Petterson (2000) and Glencross (unpublished).

2.2.2 *Protein classes*

The protein composition of lupin grains is regarded highly as a nutritional source for terrestrial domestic animals, considered comparable to that of soybeans (van Barneveld, 1999). Three of the key protein fractions found in lupins, the albumins, the globulins and the prolamines, are all, rich in glutamic acid, aspartic acid, arginine and leucine. The predominant proteins are a class of globulins referred to as the conglutins, which comprise about 85% of the total protein content (Blagrove and Gillespie, 1975). There are three classes of conglutins in lupin protein, all of which are similar sized molecules and have similar properties to other storage proteins found in field peas, soybean and other legumes. The albumin fractions of the lupin protein contain a more favorably comparable amino acid composition, relative to the amino acid composition of fish meals (Todorov et al., 1996). Overall, there is a high degree of homology in protein classes between lupins and soybeans, with both grains containing high levels (>80%) of globulins, moderate levels of albumins (10 to 20%) and essentially no glutelins (Gueguen, 1983).

2.2.3 *Amino acids*

The amino acid profile of the protein content of lupin meals compares favorably with that of soybean meal, being high in arginine, lysine, leucine and phenylalanine (Table 2.2). The notable limitation of lupin meals is the comparative deficiency of methionine and cysteine. Recent breeding efforts have been

Table 2.2 Amino acid composition (g amino acid / 16 g N) of lupin and soybean protein

Amino acid	*L. angustifolius*	*L. albus*	*L. luteus*	Soybean
Arginine	11.62	12.20	11.30	5.42
Cysteine	1.36	1.34	2.28	n/a
Histidine	2.57	1.86	3.30	2.46
Isoleucine	3.91	3.80	2.70	4.51
Leucine	6.61	6.90	7.89	6.81
Lysine	4.66	4.75	5.35	5.66
Methionine	0.72	0.66	0.70	1.28
Phenylalanine	3.65	3.85	4.04	3.60
Threonine	3.54	3.29	3.51	3.56
Tryptophan	1.00	0.97	n/a	1.35
Tyrosine	3.66	4.26	3.10	1.67

Data derived from Tacon (1990); Petterson et al. (1998); van Barneveld (1999).

directed towards improving the levels of methionine in *L. angustifolius*. Of note though, are the higher total levels of methionine, cysteine and lysine in the seed of *L. luteus*, concomitant with a higher level of protein. The level of methionine in *L. albus* is between that observed in the other two key lupin species. When examined on a basis proportional to the protein content the levels of methionine and threonine compare more favorably, though notably lysine levels are still quite low. Considerable variability has also been observed in the relative availabilities of each of the amino acids from lupin protein when fed to pigs (van Barneveld, 1999).

2.3 Carbohydrates

The carbohydrate content of lupin seed is quite different to that of most legumes (van Barneveld, 1999). The seed is charactersied by possessing high levels of both soluble and non-soluble non-starch polysaccharides (NSP). This group of carbohydrates forms primarily the structural polysaccharides of the seed, though some are considered as non-structural. In addition, starch is essentially non-existent in contrast to most other legume seeds (Petterson, 2000).

2.3.1 Starch and free sugars

Lupins are typically low in starch, with most species containing less than 15 g/kg DM in the seed. Little variability appears to exist in the levels of starch between the species and cultivars.

The free sugar content of both *L. angustifolius* and *L. albus* whole-seed meals is dominated by both glucose and galactose, each at about 30 to 40 g/kg DM. Smaller quantities (8 to 10 g/kg DM) of mannose are also present in the whole seed. While these free sugars are found in both the seed coat and kernel, the bulk of them are found in the kernel (van Barneveld, 1999).

2.3.2 Non-starch polysaccharides

The non-starch polysaccharides (NSP) constitute the major portion of the carbohydrate fraction of all lupin species. The seed coats (hulls) in particular are high in cellulose, hemicellulose and pectins (Brillouet and Riochet, 1983). The actual composition of NSP differs between the species and cultivars, though their structures are conserved (Cheung, 1990). Total NSP levels of lupin seeds are typically about 400 g/kg DM, essentially double that of soybean meal (217 g/kg DM), peas (*Pisum* sp.) (180 g/kg DM) and faba beans (*Vicia* sp.) (190 g/kg DM) (van Barneveld, 1999).

The hemicellulose content of the crude fibre was shown to be proportionally greater in lupins than in other legumes such as peas, faba beans and soybeans in which the cellulose content comprised a greater proportion of the fibre. Notably, a greater proportion of the hemicellulose is present in the kernel, with the majority of the cellulose in the seed coat (van Barneveld, 1999).

A further group of polysaccharides, the pectins are comprised primarily of β-(1,4)-galactan, which itself is comprised of sub-units of L-rhamnose, L-arabinose, D-galactose and galacturonic acid (Carre et al., 1985).

The polysaccharide group of the lignins is comparatively low in lupins compared to legumes such as soybeans and faba beans, though at a similar level to that of peas (12 g/kg DM) (van Barneveld, 1999).

2.4 Lipids

2.4.1 Crude lipid

The fat content of lupins varies considerably between the different species and even cultivars. Typically lowest in crude fat level is *L. atlanticus* (as low as 14 g/kg DM) and highest is *L. mutabilis* (up to 230 g/kg DM). Of the three key species, *L. luteus* generally has the lowest fat levels (62 to 83 g/kg DM) and *L. albus* the highest fat levels (83 to 145 g/kg DM) (Petterson et al., 1997; Petterson, 2000) (Table 2.1).

Analysis of the crude lipid composition identified that triacylglycerides made up 71.1%, phospholipids 14.9%, free sterols 5.2%, glycolipids 3.5%, sterol and wax esters 0.5%, free fatty acids 0.4%, with hydrocarbons and unidentified waxy material each contributing about 0.4% (van Barneveld, 1999; Petterson, 2000).

Table 2.3 Fatty acid composition (% of total fatty acids) of lupin and soybean

Fatty acid	*L. angustifolius*	*L. albus*	*L. luteus*	Soyabean
16:0	11.0	7.8	4.8	10.3
18:0	3.8	1.6	2.5	4.5
18:1n-9	38.2	53	21	23.9
18:2n-6	37.1	17.2	47.3	51.8
18:3n-3	5.3	9.5	7.5	6.5
20:0	0.9	1.2	2.7	-
20:1n-9	0.3	4.3	1.8	-
22:0	1.9	3.9	7.1	-
22:1n-11	-	1.9	0.8	-
Total saturates	17.6	14.5	17.1	14.8
Total monounsaturates	38.5	59.2	23.6	23.9
Total polyunsaturates	42.4	26.7	54.8	58.3
n-3 (omega-3)	5.3	9.5	7.5	6.5
n-6 (omega-6)	37.1	17.2	47.3	51.8

All other fatty acids had levels less than 0.5% in all species presented. Data derived from Tacon (1990); Petterson et al. (1998); and van Barneveld (1999).

2.4.2 Fatty acids

The general fatty acid content of the lipid in lupins is typical of that of most legumes, being high in mono-unsaturated and poly-unsaturated fatty acids (PUFA). Notable fatty acids include high levels of both oleic (18:1n-9) and linoleic (18:2n-6) acids. (Table 2.3). Palmitic and linolenic acids also form a substantial component of the total fatty acids (> 5%). Essentially there are no other PUFA in lupin fatty acids other than that provided by either both linoleic and linolenic acids.

L. luteus has the highest levels of the PUFA, approaching the composition seen in soybean oil. The composition *of L. albus* has the lowest PUFA levels, being typically higher in oleic acid that either *L. angustifolius or L. luteus* but lower in linoleic acid. However, highest levels of n-3 (omega-3) fatty acids are found in *L. albus* (9.5%) followed by *L. luteus* (7.5%), both of which were higher than that observed in soyabean oil (6.5%). The highest level of n-6 fatty acids was in *L. luteus* (47.3%) followed by *L. angustifolius* (37.1%), with the lowest levels in *L. albus* (17.2%).

2.4.3 Sterols and wax esters

Low levels of both sterols and wax esters have been identified in lupin lipids. Free sterols have been identified to comprise 5.2% of the total lipids in the seed. Acylated sterols and wax esters have also been identified, though at one-tenth the level of that of free sterols (0.5%). Further unidentified waxes made up 0.4% of the total lipid (Petterson, 2000).

2.4.4 Carotenoids

A range of carotenoids have also been identified in lupin seed and kernel meals with total carotenoid levels varying from 9 mg/kg DM in *L. luteus* whole-seed to 35 mg/kg DM in *L. angustifolius* (cv. Gungarru) (Howieson and Potts, 2001). While the primary carotenoid required by most aquaculture species, astaxanthin, is absent from lupins, several other carotenoids species are present. The majority of these are hydroxycarotenoids of the lutein family, comprising about 66% of all carotenoids found in *L. angustifolius* whole-seed. About a third of all carotenoids in *L. angustifolius* are β-carotene, though in *L. luteus* this is reduced to about 20%.

2.5 Mineral content

Key minerals in lupin seeds include calcium, magnesium, phosphorus, potassium, sodium and sulphur. Calcium levels in whole-seeds range from an average of 2.2 g/kg DM in *L. albus* to 2.4 g/kg DM in *both L. angustifolius* and *L. luteus*. Phosphorus ranges from an average of 3.3 g/kg DM in *L. angustifolius* to 5.7 g/kg DM in *L. luteus*. Potassium levels range from 8.9 g/kg DM in *L. angustifolius* to 10.8 g/kg DM in *L. luteus*. Levels of sulphur range relatively more so from levels of 2.5 g/kg DM in *L. angustifolius* to 5.1 g/kg DM in *L. luteus*. All of the mineral levels are quite variable and are reportedly quite dependent on the soil type on which the plant was grown (Petterson, 2000).

2.6 Vitamin content

A range of endogenous vitamins have been reported within lupins. Reported in *L. angustifolius* whole-seed meal have been β-carotene (3.9 mg/kg DM), thiamin (5.9 mg/kg DM), riboflavin (3.1 mg/kg DM), biotin (0.04 mg/kg DM), folate (0.4 mg/kg DM), choline (3.4 g/kg DM), niacin (40 mg/kg DM), pantothenate (1.8 mg/kg DM) and α-tocopherol (2.4 mg/kg DM) (Petterson, 2000). However, recent evaluations of *L. angustifolius* (cv. Gungarru) have reported that higher levels of α-tocopherol have been found, in some instances exceeding 4.4 mg/kg DM (Petterson, 2000).

2.7 *Anti-nutritional content*

Lupins are typically low in anti-nutritional factors, though a range of various substances have been reported. Traditionally, lupins were not considered a viable feed grain because of inherently high alkaloid levels in the grain. However, selective breeding over the last forty years has resulted in the development of low alkaloid varieties that now contain less than 0.6 g/kg DM of alkaloids, with the cultivars of some species having levels consistently less than 0.1 g/kg DM. Other potential anti-nutritionals present in lupins include oligosaccharides, phytate, saponins, tannins and protease inhibitors, though notably most of these are usually at levels not considered influential (Table 2.4). Notably, lectins have not been detected in lupins.

Table 2.4 Anti-nutrient levels of various lupin species and soy meal per kilogram (as received)

Anti-nutrient (g/kg)	*L. angustifolius* (whole seed)	*L. luteus* (whole seed	*L. albus* (whole seed	*L. angustifolius* (Kernel meal)	Soybean mea (Defatted)
Trypsin inhibitor	0.12	0.16	0.08	n/r	3.11
Alkaloids	< 0.20	< 0.50	< 0.10	< 0.12	n.d.
Oligosaccharides *	41	89	66	77	52
Phytate	5.0	9.3	5.7	n/r	15.9
Saponins	0.57	-	n.d.	n/r	n/r
Tannins	0.10	0.30	0.20	n/r	n/r

*sum of raffinose, stachyose and verbascose. n.d. : not detected. n/r : not reported. Data derived from Petterson et al. (1997).

2.7.1 Alkaloids

Present levels of alkaloids in *L. angustifolius* are usually less than 200 mg/kg. Wild-type varieties, still found in their countries of origin, may contain from 5,000 to 40,000 mg/kg of alkaloids (Harris and Jago, 1984). These alkaloids are generally bicyclic, tricyclic or tetracyclic derivatives on the molecule quinolizidine (Petterson 2000). Composition of the alkaloids in *L. angustifolius* is dominated by lupinine (42-59%), 13-hydroxylupanine (24-

45%), and angustifoline (7-15%). Other alkaloids comprise less than 2% of the total (Petterson, 2000).

Though there are no reports of problems directly attributed to alkaloids in the diets of fish, levels of alkaloids >1000 mg/kg have been reported to cause palatability problems with pig diets.

2.7.2 Oligosaccharides

The oligosaccharides of lupins are generally α-galactosyl homologues of sucrose. Of these oligosaccharides, lupins contain significant amounts of the raffinose, stachyose, verbascose and sucrose families. Raffinose has a single galactose moiety linked to a sucrose molecule, while stachyose has two and verbascose three (Petterson, 2000). The reported levels of each of the oligosaccharides in lupins varies, not only between species and cultivar, but also depending on methods of analysis (Petterson, 2000) (Table 2.4 and 2.5).

For some animal species the oligosaccharides are regarded as anti-nutritionals. Recent enzyme-supplementation technology is addressing aspects of this in both pig and poultry nutrition (Castañón et al., 1997; Gdala et al., 1997). Though the utilisation of these nutrients has not been well defined in fish, studies with pigs and poultry have shown that oligosaccharides are indigestible in the stomach or small intestine, primarily due to a lack of the enzyme α-galactosidase (EC 3.2.1.23) (Gdala et al., 1997). The level of α -galactosides in lupins ranges from 70 to 120 g/kg DM (Trugo and Almeida, 1988). High levels of raffinose oligosaccharides have been reported to present some negative nutritional effects, some of which may be applicable to fish. These include; (a) interference with the digestion of other nutrients, (b) osmotic effects of oligosaccharides in the intestine and (c) anaerobic fermentation of the sugars resulting in increased gas production (van Barneveld, 1999).

Table 2.5 Oligosaccharide content and composition of defatted soybean and lupin meals. Data derived from van Barneveld (1999), Petterson (2000)

	L. albus	*L.angustifolius*	*L. luteus*	Soybean meal
Oligosaccharides (g/kg DM)	66	41	89	52
Raffinose (g/kg DM)	2 – 8	4 – 9	8 – 9	8
Stachyose (g/kg DM)	35 – 46	35 – 38	56 – 59	46
Sucrose (g/kg DM)	12 – 19	12 – 26	7 – 13	74
Verbascose (g/kg DM)	3 – 5	12 – 19	28 –31	Trace

Extraction of oligosaccharides using an ethanol extraction process was reported to remove around 70% of the oligosaccharides in both *L. angustifolius and L. albus* (Coon et al., 1990). Later work, reviewed by van Barneveld (1999), also showed that ethanol extraction significantly improved the digestion of all amino acids from both *L. angustifolius and L. albus* by pigs. This supported the hypothesis that oligosaccharides could interfere with digestion of other nutrients when fed to pigs, and suggests that the oligosaccharide content of lupins may also be influencing the nutritional value of its own protein. In contrast to work with pigs, little influence on the nutritional value of lupins by the raffinose oligosaccharides has been observed with poultry. Studies by Hughes et al. (1998) demonstrated that the ethanol extraction the oligosaccharides from *L. angustifolius* actually reduced the nutritional value to poultry. Similar results were also reported by Irish et al. (1995) when soybean meal was ethanol extracted to remove the α-galactosides of sucrose. So in contrast to that observed with pigs, it appears that lupin oligosaccharides have little anti-nutritional effect in poultry.

Ultimately the influence of the oligosaccharides on the nutritional value of lupins appears to vary on a species specific basis. What influence the lupin oligosaccharides are likely to have on fish is presently unknown though studies examining ethanol soluble carbohydrates (most likely to be oligosaccharides) from soybean meals on Atlantic salmon, have shown some antagonistic effects (Arnesen et al., 1989).

2.7.3 Phytate

The molecule inositol hexaphosphate and salt ions of this molecule are commonly referred to as phytate. These molecules tend to form insoluble complexes with calcium and /or zinc ions, which make them less available for absorption and utilisation (Petterson, 2000).

Lupins typically have low levels (~5 g/kg DM) of phytate, similar to the levels found in peas and soybean meal, and considerably less than that in rapeseed/canola meal. The commercial use of exogenous enzyme supplements has made considerable improvements to the utilisation of phytates by both pigs and poultry. The key to this is the use of the enzyme phytase (EC 3.1.3.8) which cleaves the phosphate units from the inositol base. Recent work has indicated that there may be potential for phytase use with fish diets (Carter and Hauler, 1999; Storebakken et al., 1998b). Interestingly, improved feed intakes have also been observed of Atlantic salmon when fed diets containing phytase (Carter and Hauler, 1999).

2.7.4 Saponins

Saponins are plant glycosides with a steroid or triterpenoid structure as part of the molecule. Similar to alkaloids, saponins are also a bitter tasting molecule. This means that their primary anti-nutritional basis is as a feeding deterrent. An additional effect attributable to saponins is an increase in the permeability of the small intestine mucosal cells. Trace levels of saponins have been identified in *L. albus* seeds, with slightly higher levels (500 to 800 mg/kg DM) observed in *L. angustifolius* seeds

(Ruiz et al., 1996; Frokiaer et al., 1998). Saponin levels reported in *L. luteus* are about one tenth that of *L. angustifolius,* at 55 mg/kg (Cuadrado et al., 1995). The levels of saponins in lupins are generally about one-tenth the amount of that of soyabeans, and about half that observed in field peas (Fenwick et al., 1991).

2.7.5 Tannins

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

The tannin content of lupins is contained primarily in the seed coat of the grain. However, the condensed tannin content of the seeds are generally considered so low (~ 100 mg/kg DM) that they are unlikely to cause any anti-nutritional effect (Petterson 2000). Considerably higher levels of tannins are generally found in some varieties of soybeans, field peas and faba beans (Petterson, 2000).

2.7.6 Protease inhibitors

Protease inhibitor activity, notably that of trypsin inhibitors (TI) has been reported at less than 0.3 mg/kg in *L. angustifolius* seed. Chymotrypsin inhibitor (CI) activity was reportedly higher at 0.6 mg/kg in *L. angustifolius*, *L. luteus* and *L. albus* seed (Petterson et al., 1997). These levels of protease inhibitors are very low in comparison to some other plant protein meals, particularly other legume seeds such as soybean, which has TI levels of about 60,000 mg/kg DM in unprocessed seed and about 3,400 mg/kg DM in soybean meal (White et al., 2000).

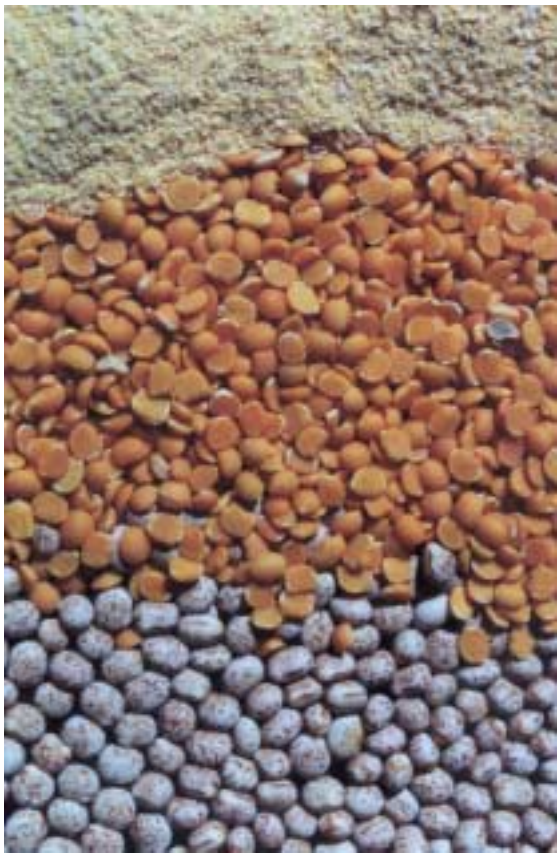

Figure 7. L. angustifolius (cv. Gungurru) seed, kernels and kernel meal (bottom to top)

3.1 Use of lupins in diets for aquaculture species

The identification and development of alternatives to fishmeal use in aquaculture diets is a high priority for improving the sustainability of aquaculture. Presently, modern intensive aquaculture is still a net fish user rather than producer (New and Csavas, 1993; Tacon, 1996). This practice questions both the reliability of aquaculture as a food provider, and also the long-term sustainability of these industries. To improve reliability, one option has been to increase the use of alternative terrestrially derived, non-food grade protein resources in intensive aquaculture diets.

A range of alternative protein resources from various sources have been identified and evaluated. While many of these resources are good, viable options for reducing fishmeal use in aquaculture diets, there are some concerns over the risks associated with using some resources, such as the potential for transmission of disease, the use of genetically modified organisms as feed ingredients and the environmental impacts associated with the use of particular ingredients.

Lupins are one of several plant protein resources that have been shown to provide sound nutritional value to a range of aquaculture species. As with many ingredients, these feed resources have their strengths and weaknesses. In comparison to other plant protein resources, the potential of lupins is equaled perhaps only by soybean meals, which are presently widely accepted and used in the aquaculture feeds sector.

While there are many similarities in the way that aquaculture species deal with lupins, when they have been included in their diet, there are specific nuances with each aquaculture species and each lupin variety that influence the relative value of lupins as a feed ingredient in the aquaculture sector. The remainder of this review examines these differences on a species by species basis.

Figure 8. Red seabream have shown good capacity to use lupin meals in their diet.

3.2 Defining the value of lupins to aquaculture species

There are several key facets to determining or placing a nutritional or biological value on a feed ingredient. Principal to this is defining the amount of nutrients that an animal can obtain from a particular substance through its digestive processes. For an animal to obtain value from any particular substance or ingredient, the level of protein and energy derivation from that ingredient needs to be defined. Only when these key parameters have been defined can the true value of an ingredient to an animal be determined.

3.2.1 Assessment of nutritional value

As previously mentioned, the key to the assessment of any new feed meal is the determination of its relative nutritional value. Typically this has been determined through digestibility studies, particularly those adopting either ingredient substitution or reference diet substitution methods (Cho, 1991; Aksnes et al., 1996; 1998; Sugiura et al., 1998; Kaushik, 1998b). In this style of study an ingredient is substituted into a reference diet either as a replacement of a proportional part of the total composition of the diet (diet substitution method), or where it replaces the inclusion of a specific, well calibrated, homogeneous ingredient such as vitamin-free casein or enzymatically hydrolysed casein, which is also included in a reference diet. The diets are then fed to the test animals and faeces collected. The method of faecal collection varies considerably, and there is some debate on the most valid method (Aksnes et al., 1996; 1998; Allan et al., 2000).

A secondary way by which many feed ingredients and/or diets are now being evaluated is by *in vitro* assessment methods (Robaina et al., 1995; Carter et al., 1999; Alarcon et al., 1999). *In vitro* assessment provides opportunities for increasing the range and number of samples examined, by significantly reducing the cost of assessment relative to the *in vivo* techniques. The key to the *in vitro* analysis techniques has been the assessment of protease activity or inhibition thereof, in a controlled simulated digestive environment. A range of methods has been developed, with a similar range of efficacies. The specific relevance of such assays is also open to conjecture, with mixed conclusions on the comparison of *in vitro* with *in vivo* data (Robaina et al., 1995; Carter et al., 1999; Alarcon et al., 1999).

An increasing amount of modern aquaculture feeds are now being formulated on a digestible protein and energy basis (Kaushik, 1998a; Burel et al., 1998; Williams, 1998). This trend is consistent with the manner in which most pig diet rations are formulated, but is still not as technically advanced as modern poultry rations which are usually formulated based on the precise requirements for specific amino acids and the metabolisable energy value of each of the feed ingredients (Hughes, 1988; Edwards and van Barneveld, 1998).

The advantages presented by development of diets on a digestible or metabolisable nutrient basis are numerous. Not only could there be a potential cost reduction, but there is also a potential reduction of waste outputs from aquaculture (Cho, 1991; Azevedo et al., 1998; Vielma et al., 2000).

In accordance with the importance of defining the nutritional value of an ingredient on its value to a particular species, where this information has been available, it has been presented preliminary to the remainder of the information available for each species examined in this review. This approach has been undertaken to allow a more objective assessment by the reader of the relative merits and meaning of the remaining studies reported.

3.2.2 Assessment of biological value

Assessment of the biological value of a feed ingredient differs from that of the nutritional value in that it also encompasses any influence an ingredient may have on feed intake and it also may examine the metabolic cost of an ingredient for tissue accretion. There is considerable debate over the most valuable way in which to determine biological value of an ingredient, though most involve the serial inclusion of increasing amounts of the test ingredient into the animal's diet. Key parameters to the assessment of the biological value focus on growth, often best measured as a function of either nitrogen or energy retention by the animal (Kaushik, 1998a; Medale et al., 1998; Williams, 1998).

One such style of studies are the summit-dilution style experiments (Allan and Rowland, 1998; Sarac et al., 1998; Williams, 1998). In these studies, the test ingredients are serially substituted into a basal reference diet, with a concomitant series of diets also being provided, where an inert filler of no nutritional value is also substituted into diets at similar inclusion levels. These inert filler diets being provided as relative negative control treatments. None of the diets are balanced for either nitrogen or energy. To avoid dietary intake compensation for nutritional inadequacies, the diets in this style of study are usually fed on a pair-fed restricted basis. This style of study has merits in that it allows an objective assessment of the relative value of the ingredient at a particular inclusion level, and information on how well that animal metabolically deals with that ingredient. Information on how well the ingredient is assimilated can also be objectively determined. For studies of this design to be useful, concomitant trials with another, well standardised ingredient are also important as a positive control. Effectively this also allows referencing of the test ingredient against a standard of some description. In studies where this experiment design has been used, fishmeal has often been used as the reference ingredient (Allan and Rowland, 1998; Sarac et al., 1998; Williams, 1998). However, the summit-dilution methods weakness is that it does not allow the determination of the ingredients influence on feed intake, nor the practical considerations of the influence of the ingredient in diets of equal nutritional content.

In other studies, the serial inclusion of an ingredient has been undertaken where the diets have also been balanced on an iso-nitrogenous and iso-energetic basis (Robaina et al., 1995; Burel et al., 1998). Typically, the diets in these studies have been fed to apparent satiety or to an approximated daily ration, thereby allowing the determination of influences of the ingredients on feed intake parameters. While this style of study has potentially more practical value, the inclusion of a negative control is important to

demonstrate that the relative inclusion of a specific ingredient is actually contributing to the value of the diet, rather than acting as a filler in an over specified diet. Unfortunately, the use of appropriate controls in such studies has been frequently lacking, in many instances making interpretation of the ingredient value from such trials a little more ambiguous.

Despite the limitations to many of the biological value studies reported, the published data have in most instances provided some important information, on the usefulness of lupins in the diets of aquaculture species. As such, this has allowed the assessment of the speculated value of the included ingredients. Accordingly, information of this type has been included within this section on biological value, though the limitations are discussed where appropriate.

To allow an objective assessment of the biological value of a diet or ingredient there are several key parameters that need to be defined, essential are the nitrogen and energy retention efficiencies. These two parameters are often referred to as protein productivity value (PPV) and energy retention value (ERV) and are usually expressed as a percentage. Essentially these parameters define the relative amount of each nutrient/energy that is actively derived from a particular diet or ingredient. The greater the efficiency, the greater the value being derived. Similarly, the determination of these parameters from serial inclusion diets also allows a critical appreciation of the influence that a particular ingredient has on the biological value of a diet (Kaushik 1998a; Medale et al., 1998). Some recent studies are now also reporting phosphorus retention efficiencies (Burel et al., 1998).

A derivation of the nitrogen and energy retention efficiency parameters is the Apparent Biological Value (ABV) term. ABV is determined by expressing the retention efficiency parameter as a function of the amount of ingested digestible nutrient or energy. In the case of nitrogen/protein, this would therefore be determined by calculating the retention of nitrogen as a function of total digestible nitrogen consumed. The ABV value in this essence is a more technically accurate way of assessing the true biological value of an ingredient to the growth of an animal.

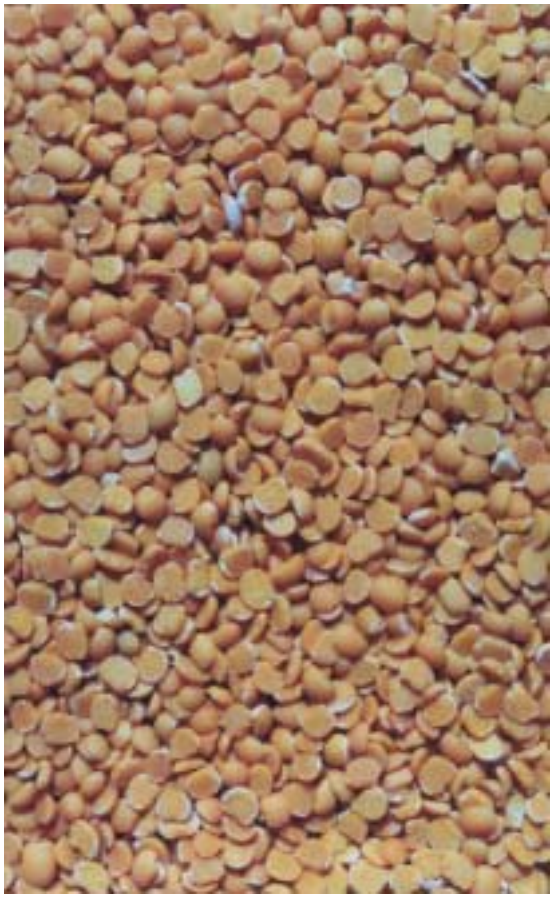

Figure 9. Kernels of L. angustifolius

4.1 Salmonids

Salmonids have been the most extensively studied aquaculture species for both nutritional research in general and the usefulness of lupins to an aquaculture species. Generally, the rainbow trout (*Oncorhynchus mykiss*) has been the primary species studied, though a growing volume of work has also been reported with the Atlantic salmon (*Salmo salar*).

4.1.1 Rainbow trout

There are few studies that have implicitly studied the nutritional value of lupins to rainbow trout. The earliest reported study, was that by Hughes (1988) who examined the nutritional value of *L. albus* whole-seed meal. In this study the apparent digestibility of protein was reported at 85.2%, and was reportedly higher than that of full-fat soybean meal (79.5%). Apparent energy digestibility of the *L. albus* whole-seed meal was 64.0% and was reportedly lower than that of the full-fat soybean meal (74.7%). The differences in digestible energy were attributed to the 61 g/kg DM higher level of lipid in the full-fat soybean meal. Similarly the metabolisable energy of the full-fat soybean meal (3998 kcal/kg DM) was also higher than that of the *L. albus* whole-seed meal (2981 ± 135 kcal/kg DM), though this too is probably reflective of the considerably higher lipid levels of the full-fat soybean meal.

A subsequent, and more thorough study by Morales et al. (1994) examined the apparent digestibility characteristics of a range of ingredients when fed to rainbow trout. The diets in this study were formulated to include *L. albus* meal (cultivar or processing state not identified), corn gluten meal, casein and cottonseed meal at 40% of the total dietary protein as partial replacements for the fishmeal portion of iso-nitrogenous and iso-energetic diets. Additional diets in this study included a reference diet with fishmeal as the only protein resource, and another reference diet with casein as the only protein resource. The inclusion of fishmeal as the only protein resource in the reference diet allows specific assessment of the nutritional value of the protein content of each of the test diets and their ingredients as used in this study. The key importance of the 100% casein diet is that it should allow identification of endogenous protein losses by the trout fed this diet. Furthermore, the evaluation of the digestibility of the diet where casein was included as 40% of the total protein also allows some relative estimates of the true protein digestibilities of each of the test ingredients.

Assessment of the apparent digestible characteristics of each of the diets revealed that the apparent digestible dry matter of the lupin diet was lowest, as was the apparent digestibility of its organic matter and energy content. It is suspected that these observations are reflective of the relatively high levels of non-starch polysaccharides in the *L. albus* meal. Indeed evaluation of the digestibilities of the NFE and carbohydrate contents of the diet clearly support this, with the lupins having the lowest NFE and carbohydrate apparent digestibilites of all the ingredients evaluated in this study, by a considerable margin.

The apparent protein digestibility of the lupin diet was higher than that of the cottonseed meal diet, but not as high as that of the corn gluten meal diet. It was though very comparable to the apparent protein digestibility of the fishmeal based reference diet (Table 4.1). The diet with the highest apparent protein digestibility was the 100% casein diet (~97%). The 40% casein diet also had a high apparent protein digestibility, though notably it was about the same as that of the corn gluten meal diet (Table 4.1). Based on the determined digestibility value of the fishmeal protein it was calculated that the casein and corn gluten had apparent protein digestibilties of about 97% with the next highest being the *L. albus* meal (85%), which was slightly higher than that of the fishmeal (84%).

Table 4.1 *Apparent digestibility (%) of key dietary nutrients from diets based on a range of protein resources fed to rainbow trout. Data derived from Morales et al. (1994). Full details of diet composition and growth performance detailed in subsequent sections.*

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

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

Gomes et al. (1995) also evaluated the nutritional value of a suite of plant protein meals in rainbow trout. Included in this study were a range of plant legume meals, including *L. angustifolius* whole-seed meal, pea (*Pisum sativum*) seed meal, faba bean (*Vicia faba*) meal, full-fat toasted soybean meal and full-fat micronised soybean meal (Table 4.2). A range of other cereal, marine and animal meals were also included. Of the plant legume protein meals, full-fat micronised soybean meal had the highest apparent dry matter digestibility (86.4%) and *L. angustifolius* seed meal the lowest (63.3%). The apparent dry matter digestibility values of pea seed meal (66.6%) and faba bean meal (66.1%), were similar to that of and *L. angustifolius* seed meal. Apparent protein digestibility of the legume meals was also highest in full-fat micronised soybean meal (96.3%) and the lowest faba bean meal (80.2%). The apparent protein digestibility of *L. angustifolius* seed meal was the highest of the unprocessed whole-seed meals (85.5%). No significant differences were evident between the three whole-seed legume meals, though the soybean meals had significantly higher protein digestibilities.

The apparent energy digestibilities of the plant legume meals ranged from 59.2% to 90.7%. The highest was that of the full-fat micronised soybean meal (90.7%) and the lowest that of the pea seed meal (59.2%). The apparent energy digestibility of the *L. angustifolius* seed meal was similar to the other whole-seed legume meals (61.2%). No significant differences were evident between the three whole-seed legume meals, though the soybean meals had significantly higher apparent energy digestibilities. The marked difference between the soybean meals and that of the other legume meals clearly demonstrates the value of processing the seeds from these plants to improve their nutritive value to fish. Though notably the cooking (toasting) of the meals significantly reduces the protein value of the meal (Table 4.2).

This study in particular is interesting in that it is one of the few accounts where the nutritive value of the protein in a lupin (notably the whole-seed meal in this case) has been shown to be less than that of the soybean meals also used in the same study.

Table 4.2 *Digestibility values of a range of protein resources, including L. angustifolius whole-seed meal, fed to rainbow trout. Data derived from Gomes et al. (1995).*

	Dry matter digestibility (%)	Protein digestibility (%)	Energy digestibility (%)
Fishmeal	78.0	86.6	69.7
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

The most comprehensive account examining the nutritive value of a lupin meal to rainbow trout would be that reported by Burel et al. (2000a) who examined the value of extruded *L. albus* kernels, extruded peas and both solvent and heat-treated rapeseed (*Brassica* sp.) meals. The apparent digestible dry matter, protein (as N x 6.25), energy and phosphorus of these ingredients were studied using the diet substitution assessment method (Aksnes et al., 1996). Key findings from the work of Burel et al. (2000a) was the significantly higher protein digestibility of *L. albus* kernel meal in comparison to the pea and rapeseed meals. In addition, the energy digestibility of the *L. albus* kernel meal was also significantly higher than

that of pea 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. Low levels of starch in both the *L. albus* kernel and rapeseed meals support that limited dietary energy would be obtained from carbohydrates in these ingredients, with the majority of the energetic value being derived from their protein content. However the 100 g/kg level of lipid in the *L. albus* kernel meal probably also provided considerable digestible energetic value.

Also of note was the significantly higher phosphorus digestibility of the *L. albus* kernel meal in comparison to both the pea and rapeseed meals (Table 4.3). The higher phosphorus digestibilities are of particular note in this species, given the predominance of its culture in freshwater systems where phosphorus is often perceived as a undesirable waste nutrient (Ervik et al., 1997; Elberizon and Kelly, 1998). While the data of Burel et al. (2000) provides a good foundation for the further assessment of the phosphorus output reduction potential of some meal types, the determination of retention values for both phosphorus and nitrogen would have added to the value of these findings. This would have allowed the subsequent capacity to determine nutrient waste budgets on an ingredient specific basis.

There were no significant differences in the dry matter digestibilities of any of the ingredients in this study (Table 4.3).

Table 4.3 *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	928	937	915
Crude protein (g/kg DM)	260	434	431	433
Crude fat (g/kg DM)	4.5	100	48	9
Ash (g/kg DM)	33	46	79	82
NFE (g/kg DM)	612	348	379	391
Phosphorus (g/kg DM)	4.4	5.4	14.9	15.6
Nutrient Apparent Digestibility				
Dry matter (%)	66.3	69.7	70.8	66.6
Protein (%)	87.9	96.2	90.9	88.5
Energy (%)	68.9	77.0	76.4	70.0
Phosphorus (%)	42.6	61.9	26.4	41.8

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

In the initial work on lupins by Hughes (1988), the biological value of whole-seed *L. albus* meal (CP: 380 g/kg DM, CF: 131 g/kg DM; cultivar not stated) was also compared against full-fat soybean meal as a feed ingredient for rainbow trout (Table 4.4). The study used either of two basal diet formulations, with the first experimental diet based primarily on fishmeal, with some soybean meal and the second primarily on full-fat soybean meal.

In the first experiment, utilising the fishmeal based diet, *L. albus* whole-seed meal was substituted for the full-fat soybean meal included in the formulation at 85 g/kg (level of diet DM not defined). After a 14-week feeding study, fish increased from 2.4 g initial weight to 18.9 g final weight in both treatments. Feed conversion in both cases was 1.18:1 and 1.20:1 for full-fat soybean meal and *L. albus* whole-seed meal respectively (Table 4.4).

In the second experiment, the diet based primarily on full-fat soybean meal was altered with the substitution of *L. albus* whole-seed meal, for all of the 400 g/kg inclusion level of full-fat soybean meal. In this experiment, the fish increased in weight from 2.4 g to 16.6 ± 0.2 g when fed the full-fat soybean meal diet, and 17.4 ± 0.9 g when fed the *L. albus* whole-seed meal diet. Differences in growth were not significantly different. However, feed conversion by fish fed the *L. albus* whole-seed meal diet was slightly better than that reported for the full-fat soybean meal diet (Table 4.4).

The results from this study supported either equal nutritional value of both grain resources at either inclusion level, or gross over-specification of the original basal diet. No indication was given of the gross nutritional specifications of any of the experimental diets, nor were there any negative controls in this study, these limitations preventing any further critical evaluation of the outcomes of this work.

In a subsequent study, Hughes (1991) also reported the influence of removing the seed coat of *L. albus* grain (*L. albus* CP: estimated at 39%, cultivar not defined). In this second study, a diet based on full-fat soybean meal was compared against four other diets, two each containing either *L. albus* whole-seed or the kernel meal (Table 4.5). In this study Hughes (1991) also added soybean oil to one each of the treatments examining either *L. albus* whole-seed or kernel meal, in an attempt to balance the dietary energy content between the diets. Diets were formulated to be approximately iso-nitrogenous though marked energetic differences were present between those diets with and without the supplemented soybean oil. The full-fat soybean meal diet was of an equivalent energetic value to the *L. albus* whole-seed and kernel meal diets with the supplemental soybean oil.

Growth data provided good support for the value of removing the seed coat of lupins to improve their biological value. Fish fed either of the *L. albus* kernel meal diets outperformed all other treatments, including the full-fat soybean meal diet fed trout, with both superior growth rates and FCR values (Table 4.5). Retrospectively, this result is not surprising given that the diets were formulated to be iso-nitrogenous on a gross basis only. Digestibility data from Hughes' (1988) earlier study

supported that a markedly higher apparent digestibility for protein from the *L. albus* whole-seed meal over that of soybean meal, would have meant that a greater amount of protein would have been available to the fish from those diets. This observation may also possibly indicate that the diets for these fish may have been under-specified for protein and/or energy. As with this researcher's earlier work (Hughes 1988), this problem could have been averted by the inclusion of single negative control treatment.

Table 4.4 *Utilisation of L. albus whole-seed meal by rainbow trout, when substituted for full-fat soybean meal in two separate experiments. Data derived from Hughes (1988).*

	Reference 1	Lupin 1	Reference 2	Lupin 2
	Experiment 1		Experiment 2	
Ingredients				
Fishmeal	340	340	80	80
Soybean meal (full fat)	85	-	400	
L. albus whole-seed meal	-	85	-	400
Corn gluten	80	80	120	120
Blood meal	55	55	50	50
Brewers yeast	55	55	100	100
Cottonseed meal	112	112	-	-
Fish oil	35	35	40	40
Soybean oil	68	79	-	52
Molasses	120	120	-	-
Wheat middlings	22	11	122	70
Dried whey	-	-	60	60
Remains (uniform across treatments)	28	28	28	28
Diet proximate specifications				
	NOT PROVIDED		NOT PROVIDED	
Fish performance criteria				
Initial weight (g)	2.4	2.5	2.4	2.4
Final weight (g)	18.9	18.9	16.6	17.4
DGC (%/d)	4.42	4.36	4.04	4.17
FCR (g fed / g gain)	1.18	1.20	1.36	1.25

Table 4.5 *Utilisation of soybean and L. albus whole-seed and kernel meals by rainbow trout. Data derived from Hughes (1991).*

	FFSM	*L35*	*L35SO*	*L39*	*L39SO*
Diet ingredients (g/kg)					
Full-fat soybean meal	400	-	-	-	-
L. albus meal (35% protien)	-	400	400	-	-
L. albus meal (39% protein)	-	-	-	400	400
Soy oil	-	-	52	-	52
Wheat middlings	96	96	44	96	44
Remains (uniform across treatments)	504	504	504	504	504
Diet proximate specifications					
Crude protein (g/kg DM)	436	415	408	436	424
Crude fat (g/kg DM)	159	101	157	106	154
Gross ash (g/kg DM)	84	79	73	82	75
Gross energy (MJ/kg DM)	22.12	20.79	22.09	20.98	22.09
Fish performance criteria					
Initial weight (g)	13.3	13.3	13.3	13.3	13.3
Final weight (g)	36.8	36.1	36.1	37.7	38.6
DGC (%/d)	1.14	1.11	1.11	1.17	1.20
FCR (g fed / g gain)	1.45	1.45	1.46	1.39	1.36

FFSM: full-fat soybean meal diet, L35: *L. albus* (35% protein, suspected whole-seed meal) diet, L35SO: *L. albus* (35% protein) meal diet with added soybean-oil, L39: *L. albus* (39% protein, suspected kernel meal) diet, L39SO: *L. albus* (39% protein) diet with added soybean-oil

De la Higuera et al. (1988) was also one of the first to have evaluated the biological value of *L. albus* whole-seed meal (cv. *Multolupa*). In this study the *L. albus* whole-seed meal was either cooked or uncooked before being included at several inclusion levels in diets fed to rainbow trout. Each of the diets was formulated to be iso-nitrogenous and iso-energetic, though on a gross basis only.

Fish from all of the treatments in which lupins were included, grew considerably poorer than those fed the reference diet in this study (Table 4.6). Relationships between growth and the inclusion level of either the cooked or uncooked *L. albus* whole-seed meal were poor. Stronger relationships were observed between growth and gross dietary energy content. Re-evaluation of the data from this study based on digestible energy and protein values would probably clarify the observations considerably. Nitrogen retention of fish fed to satiety was uniform (32.2% - 34.5%) from diets containing uncooked *L. albus* whole-seed meal, except at the 40% inclusion level were it dropped to only 25.1%. Nitrogen retention from diets including the cooked *L. albus* whole-seed meal was generally poorer (26.1%

- 28.5%) than that of the uncooked *L. albus* whole-seed meal, consistent with possible heat damage of the protein content (van Barneveld, 1993). A more uniform allocation of fish, based on equal initial weights, to treatments would have also improved this study.

A notable influence on feed intake by fish fed the diets containing lupins was observed relative to the fishmeal based reference diet (Table 4.6). The response did not appear proportional to the relative inclusion level of uncooked *L. albus* whole-seed meal, though it was partially related to the inclusion level of the cooked *L. albus* whole-seed meal. De la Higuera et al. (1988) suggested that the palatability issues may have been influenced by endogenous alkaloids within the *L. albus* whole-seed meal, which were reported at a concentration of 0.25 g/kg in this paper. However, a definitive explanation of why the magnitude of feed intake varied as such was not given. The variability of feed intake had considerable impact on the FCR values observed in this study (Table 4.6).

The apparent digestibilities of each of the diets were also examined in this study. There were essentially no differences in apparent protein digestibility between the reference diet and any of the diets containing the uncooked *L. albus* whole-seed meal (ADN: 81.4% - 82.9%). Cooking of the *L. albus* whole-seed meal reduced the apparent protein digestibility of the diets, with the apparent protein digestibilities of the 10% and 20% cooked *L. albus* whole-seed meal diets at 75.9% and 73.0% respectively. However, the reduction in the apparent protein digestibility of the diets was not consistent with the inclusion level of cooked *L. albus* whole-seed meal, with the diet that containing 30% cooked *L. albus* whole-seed meal having an ADN of 84.4%. It was suggested that these aberrations might have been influenced by poor fish health, though no other explanation for the differences was offered.

Figure 10. Lupins have shown good promise when fed to rainbow trout

Table 4.6 Performance of rainbow trout when fed diets including L. albus whole-seed meal. Data derived from De la Higuera et al. (1988).

		Uncooked *L. albus* whole-seed meal				Pre-cooked *L. albus* whole-seed meal			
	Reference	10%	20%	30%	40%	10%	20%	30%	40%
Diet proximate specifications									
Crude protein (g/kg DM)	460	457	455	447	457	453	439	455	445
Crude fat (g/kg DM)	100	99	95	98	87	98	105	91	97
Gross ash (g/kg DM)	126	140	134	127	125	147	140	130	124
Nitrogen Free Extract (g/kg DM)	314	304	316	328	331	302	316	324	334
Gross energy (MJ/kg DM)	20.24	19.95	19.96	20.09	19.95	19.79	19.97	19.94	20.11
Fish performance criteria									
Initial weght (g)	46.6	41.9	36.7	43.7	42.9	43.7	50.5	46.2	44.2
Final weight (g)	87.8	76.3	65.7	79.2	66.0	79.6	71.9	66.3	66.6
DGC (%/d)	2.82	2.56	2.37	2.58	1.80	2.60	1.54	1.53	1.72
Feed intake (g/fish/d)	1.73	1.49	1.22	1.47	1.27	1.75	1.36	1.34	1.26
FCR (g fed/g gain)	1.26	1.29	1.26	1.24	1.64	1.46	1.90	2.00	1.68
Nitrogen retention (%)	34.5	34.3	32.2	33.4	24.1	27.8	28.5	26.4	26.1
Protein digestibility (%)	81.6	82.9	81.9	81.4	81.8	75.9	73.0	84.4	81.0

A fishmeal replacement study was undertaken by Moyano et al. (1992), where the fishmeal in the diet was replaced on a 50%, 70% or 100% basis, by a range of plant protein resources, including a lupin meal. The diets in this study were balanced to maintain the diets on an iso-energetic and iso-nitrogenous basis. No negative controls were included to substantiate the effect of alternative inclusions on the diets with less than 100% fishmeal replacement (Table 4.7). No specific details were given to the lupin species, variety or even processing form used in the study, though the ingredient composition data of the provided suggest that it was probably *L. albus* kernel meal.

Growth performance of the fish fed the 50% fishmeal replacement diets supported that lupin meal had high-potential as a protein resource for trout. It could also be implied that the lupin meal was marginally superior to corn gluten meal, based on the performance of trout fed a diet where the 50% replacement of fishmeal was shared between both lupin meal and corn gluten meal, relative to the diet where corn gluten meal was the sole alternative protein resource (Table 4.7).

The performance of fish fed the diet where a 70% replacement of fishmeal was shared between both lupin meal and corn gluten meal suggested that the capacity of these two protein resources to fully sustain growth was slightly reduced compared to the diet with slightly lower lupin and corn gluten meal levels and with higher fish meal levels. Notably though, this reduced growth performance was primarily through a lower feed intake, as a decline in nutritive value of the diet was not observed.

Fish fed the 100% fishmeal replacement diets had considerably poorer performance than the 50% fishmeal replacement diets when fed to trout. In these diets the performance of trout was poorest when fed the diet containing lupin and corn gluten as the sole protein sources. Though interestingly, the growth data were inconsistent with the nitrogen retention, protein efficiency and feed conversion data, suggesting some aberration in feed management with the fish in this treatment. Despite the slightly lower growth of this treatment, the difference was not significant from that of any of the other 100% fishmeal replacement diets, suggesting high levels of variability within the data.

It was suggested that the poorer performance of the 100% fishmeal replacement diets was indicative of anti-nutritive factors present in the plant protein resources used. Notably, trypsin-inhibitors and oligosaccharides present in the soybean meal were suggested, though it was also noted that the same oligosaccharides were likely to be present in the lupin meals, and that physiologically they should be considered as fibre when fed to these animals. It was also noted in the study by Moyano et al. (1992) that the protein digestibility values of soybean meal and lupin meal differed considerably, with soybean having a lower protein digestibility of compared to that of *L. albus* kernel meal. It was suggested that this difference in protein digestibility perhaps explained the differences in growth observed between the 50% fishmeal replacement diets in this study.

Table 4.7 *Nutritive value of diets with a high inclusion of plant proteins when fed to rainbow trout. Derived from Moyano et al. (1992).*

	Reference	50-1	50-2	70	100-1	100-2	100-3
Diet ingredients (g/kg)							
Fishmeal	627	314	314	191	-	-	-
L. albus kernel meal	-	-	326	266	272	326	-
Soybean meal	-	-	-	-	188	-	282
Corn gluten meal	-	237	119	208	237	327	327
L-Methionine	-	-	-	-	7	6	4
Potato concentrate	-	55	-	55	82	82	82
Fish oil	-	36	36	45	60	60	60
Corn oil	60	57	15	21	15	12	48
Cellulose	163	164	53	85	-	51	59
Remains (uniform across treatments)	138	138	138	138	138	138	138
Diet proximate specifications							
Crude protein (g/kg DM)	480	469	468	467	473	471	469
Crude fat (g/kg DM)	118	119	119	121	117	119	122
Fish performance criteria							
Initial weght (g)	30.5	32.8	30.9	31.8	30.3	32.9	30.1
Final weight (g)	49.3	50.5	52.7	51.4	42.6	42.5	42.8
DGC (%/d)	1.81	1.65	2.04	1.83	1.25	0.95	1.29
Feed intake (g/fish/d)	0.45	0.45	0.47	0.44	0.40	0.44	0.39
FCR (g fed/g gain)	1.43	1.47	1.28	1.23	1.72	1.37	1.64
Nitrogen retention (%)	35.8	35.7	30.7	39.3	28.2	35.7	31.0
Protein efficiency ratio (g/g)	1.42	1.45	1.67	1.74	1.22	1.55	1.29

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

Morales et al. (1994) evaluated *L. albus* (cultivar and processing form not stated) with comparison to corn gluten, cottonseed meal and a fishmeal reference diet (Table 4.8). The diets in this study were generally formulated to include about half of the dietary protein from the plant protein resource, with the remainder being provided by fishmeal. One diet was formulated where all of the dietary protein was provided by casein. No negative controls were included in the trial to substantiate the effects of alternative inclusions on the diets.

The best growth was observed from fish fed the *L. albus* meal-based diet (Table 4.8). Poorest growth was observed from fish fed the diet with 100% of the protein provided by casein. Good growth of fish fed the *L. albus* meal diet was attributed to the combined effect of both superior feed intake and superior feed conversion, with the highest feed intake observed with this diet, as well as the lowest feed conversion ratio. Similarly, the protein efficiency ratios were also best by fish fed the *L. albus* meal -based diet.

The retention of nitrogen by fish fed the *L. albus* meal-based diet was, however, the lowest of all the experimental treatments, as was the apparent biological value of the diets. This is interesting given that it contrasts so markedly with the other performance criteria (Table 4.8).

Morales et al. (1994) considered that the better growth of fish fed the *L. albus* meal-based diet and the higher content of body fat on these animals was consistent with highly efficient energy retention by the fish fed the *L. albus* meal-based diets.

Figure 11. Lupins typically have high protein digestibilities when fed to salmonids.

Table 4.8 *Replacement of fishmeal by alternative protein resources in diets for rainbow trout. Data derived from Morales et al. (1994).*

	Fishmeal	CA100%	CA40%	CO	LU	CG
Diet ingredients (g/kg)						
Fishmeal	593.3	-	356	356	356	356
L. albus kernel meal	-	-	-	-	432.1	-
Casein	-	454.4	181.8	-	-	-
Cottonseed meal	-	-	-	316.6	-	-
Corn gluten meal	-	-	-	-	-	234.1
Pregelled starch	200	200	200	109.5	63.8	151
Fish oil	3.2	51.6	22.5	26	26	26
Corn oil	60	60	60	47.3	13	46.2
Cellulose	48.5	139	84.7	49.6	14.1	91.7
Remains (uniform across treatments)	95	95	95	95	95	95
Diet proximate specifications						
Crude protein (g/kg DM)	419.1	424.6	426.6	419.6	423.2	425.2
Crude fat (g/kg DM)	108.5	111.6	96	107.1	103.9	113.4
Gross ash (g/kg DM)	151.6	57.5	111.6	131	124.8	110.6
Nitrogen Free Extract (g/kg DM)	261.9	256.1	270.5	232.8	224.9	256.7
Gross energy (MJ/kg DM)	19.55	21.58	20.30	20.05	20.01	20.70
Fish performance criteria						
Initial weght (g)	38.53	40.53	40.1	40.48	37.48	38.17
Final weight (g)	88.26	84.59	90.82	94.16	95.12	84.03
DGC (%/d)	3.58	3.18	3.57	3.72	4.06	3.38
Feed intake (g/fish/d)	0.72	0.58	0.69	0.82	0.85	0.65
FCR (g fed/g gain)	1.00	1.17	1.06	0.93	0.92	1.05
Nitrogen retention (%)	42.7	55.2	48.9	44.2	41.8	46.4
Apparent biological value (%)	51.0	56.8	55.3	54.4	49.1	52.1
Protein efficiency ratio (g/g)	2.39	2.75	2.49	2.23	2.16	2.47

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

Table 4.9 *Diet specifications and associated trout performance criteria from fishmeal replacement diets including L. angustifolius whole-seed meal. Data derived from Gomes et al. (1995).*

	Reference	*33%*	*66%*	*100%*
Ingredient				
Fishmeal	528	405	200	-
L. angustifolius whole seed meal	-	-	30	25
Full-fat soybean meal	-	83	224	324
Faba bean meal	-	27	54	69
Pea seed meal	-	40	-	-
Maize gluten	-	132	250	380
Co-extruded pea and canola meal	-	9	-	-
Meat meal	94	-	-	-
Dextrin	308	234	172	132
L-Lysine	-	-	5	10
L-Methionine	-	-	2	2.5
Remains (uniform across treatments)	70	70	70	70
Proximate composition				
Dry matter (g/kg)	925	917	910	907
Crude Protein (g/kg DM)	431	427	447	452
Crude Fat (g/kg DM)	85	89	111	127
Gross Energy (MJ/kg DM)	21.6	22.1	22.7	23.1
Gross Ash (g/kg DM)	124	100	80	47
Digestible protien (g/kg DM)	395	385	387	385
Digestible energy (MJ/kg DM)	19.3	19.0	19.0	19.0
Fish performance				
Initial weight (g)	53.5	54.8	55.5	53.9
Final weight (g)	196.5	197.3	197.3	175
DGC (%/d)	3.65	3.61	3.58	3.24
FCR (g fed/g gain)	1.04	1.09	1.01	1.06
Feed intake (g/fish/d)	2.60	2.69	2.40	2.01
Protein efficiency ratio (g/g)	2.24	2.15	2.25	2.18

Gomes et al. (1995) also evaluated the biological value of a range of protein resources in the diet of rainbow trout. Of key comparative interest were the values of pea seed meal, faba bean meal, full-fat toasted soybean meal, full-fat micronised soybean meal and *L. angustifolius* whole-seed meal (cultivar not identified) (Table 4.9). Although these workers examined the digestibilities of each of these grains individually (see earlier sections, Table 4.3) no individual assessments were made of the ingredients on a biological value basis. Instead these workers adopted a practical approach of using a range of resources in each of the diets in an effort to replace their fishmeal portions. Three alternative diets, with progressively reduced amounts of fishmeal were tested against a reference diet (Table 4.9). Each of the diets was formulated to be iso-nitrogenous and iso-energetic on a digestible basis. There were no significant differences between the reference diet and the first two diets with 33% and 66% replacement of the total fishmeal. The *L. angustifolius* seed meal comprised only a small component of each of the 66 and 100% fishmeal replacement diets.

So although this study does not provide any direct evidence for the biological value of *L. angustifolius* seed meal, it does support the potential for the use of this ingredient in diets for rainbow trout. However, the results of this study did indicate that the fishmeal component could be replaced using a suite of ingredients, but that feed intake problems were encountered with the complete replacement of the fishmeal.

The most comprehensive work published to date evaluating lupins with rainbow trout, has been that by Burel et al. (1998) who conducted a series of studies examining the inclusion of *L. albus* kernel meal in diets for rainbow trout. The first of these studies evaluated the inclusion of *L. albus* kernel meal at 300 g/kg, 500 g/kg and 700 g/kg in diets that were designed to be iso-nitrogenous and iso-energetic. Another of the diets evaluated was a control diet in which no *L. albus* kernel meal was added (Table 4.10). The results of this study identified that *L. albus* kernel meal could be included in the diet of rainbow trout to a level of 500 g/kg with no loss in growth rate and significantly superior phosphorus retention. The inclusion of *L. albus* kernel meal to 700 g/kg however, resulted in poorer growth, feed efficiency and nitrogen and energy retention. Interestingly though, phosphorus retention improved further still with the higher inclusion level of *L. albus* kernel meal (Table 4.10). The loss in growth performance of fish fed the diets containing 700 g/kg of *L. albus* kernel meal was attributed to low feed intakes of this diet. It was suggested that high levels of *L. albus* kernel meal inclusion resulted in a loss of palatability of the diet

In a second experiment conducted by Burel et al. (1998) a further series of diets were formulated and prepared to examine the high inclusion levels of *L. albus* kernel meal. In this second study three diets contained 700 g/kg of the *L. albus* kernel meal, but with some of the diets also containing either a dietary ingestion stimulant (Finnstim™) or dietary iodine (Table 4.11). These two additional diets containing the dietary supplements were included to more

clearly define whether the poor growth observed of the trout fed the 700 g/kg diet in the first study were the result of poor feed intake, or metabolic suppression. It was suggested that the poor growth of fish fed the 700 g/kg diet was possibly a consequence of suppressed metabolic rate induced by anti-thyroidal anti-nutritional factors present in the *L. albus* kernel meal.

The results of the second study however, showed no benefit of the inclusion of the iodine or the feed stimulant, with fish fed any of the three diets containing 700 g/kg of *L. albus* kernel meal having the same level of growth, feed efficiency and nutrient retention characteristics (Table 4.11). Of note though, fish fed any of the three 700 g/kg *L. albus* kernel meal diets had significantly poorer performance attributes (growth, feed utilisation efficiency and nitrogen retention) than those fish fed the control diet. This suggested that the problem encountered in the first study with poor performance with high inclusion levels of *L. albus* kernel meal had still not been overcome or identified. A third experiment examined trout fed the control diet, the 500 g/kg and 700 g/kg diets, and a 700 g/kg diet containing Finnstim™. In this study the fish were allowed to self-regulate their own feed intake (Table 4.12). However, in this study considerably different results were obtained to those observed in the second study.

In the third study no significant differences in the growth performance of fish fed each of the treatments was observed, though feed efficiency deteriorated with increasing inclusion of *L. albus* kernel meal, and deteriorated further again with the inclusion of Finnstim™. Examination of feed intake of the two 700 g/kg diets showed no benefit from the inclusion of the Finnstim™. However, despite the deteriorating feed efficiency and energy retention with increased inclusion of *L. albus* kernel meal, considerable improvements in phosphorus retention of fish fed the diets were observed.

Figure 12. Both Atlantic salmon and Rainbow trout have been shown to accept lupin meals as a diet ingredient

Table 4.10 *Influence of inclusion levels of extruded L. albus kernel meals fed to rainbow trout. Data derived from Burel et al. (1998).*

	Reference	30% Lupin	50% Lupin	70% Lupin
Diet ingredients (g/kg)				
L. albus kernel meal	-	300	500	700
Fishmeal	530	350	205	65
Flaked corn	320	205	135	75
Fish oil	90	85	100	100
L-Methionine	-	-	-	2
Remains (uniform across treatments)	60	60	60	60
Diet proximate specifications				
Dry matter (g/kg)	940	954	941	943
Crude protein (g/kg DM)	400	409	400	393
Crude fat (g/kg DM)	161	163	185	191
Gross phosphorus (g/kg DM)	15.4	11.8	9	6.4
Gross ash (g/kg DM)	104	84	66	50
Gross energy (MJ/kg DM)	21.4	21.7	22.3	22.6
Fish performance criteria				
Initial weight (g)	23.1	23.1	23.1	23.1
Final weight (g)	90.6	108.1	94.2	53.8
DGC (%/d)	2.7	3.1	2.7	1.5
Feed intake (g/d/fish)	1.07	1.33	1.13	0.62
FCR (g fed/ g gain)	1.01	1.01	1.02	1.31
Nitrogen retention (%)	36.0	39.1	37.3	31.6
Phosphorus retention (%)	28.8	37.7	39.9	69.0

Table 4.11 *Influence of attractants and iodine supplements on high inclusion levels of extruded L. albus kernel meals fed to rainbow trout. Data derived from Burel et al. (1998).*

	Reference	Control	Attractant	Iodine
Diet ingredients (g/kg)				
L. albus kernel meal	-	700	700	700
Fishmeal	480	120	125	115
Pea meal	200	28	13	33
Pregelled starch	150	-	-	-
Fish oil	110	90	90	90
L-Methionine	-	2	2	2
Attractant	-	-	10	-
Potassium Iodide	-	-	-	65 x 10-5
Remains (uniform across treatments)	60	60	60	60
Diet proximate specifications				
Dry matter (g/kg)	866	902	877	871
Crude protein (g/kg DM)	444	449	456	459
Crude fat (g/kg DM)	170	187	185	186
Gross phosphorus (g/kg DM)	14.4	8.7	8.5	8.6
Gross ash (g/kg DM)	101	61	62	61
Gross energy (MJ/kg DM)	21.9	22.8	22.6	22.7
Fish performance criteria				
Initial weight (g)	34.0	34.0	34.0	34.0
Final weight (g)	104.2	86.4	89.5	85.3
DGC (%/d)	2.7	2.1	2.3	2.1
Feed intake (g/d/fish)	1.22	1.07	1.09	1.14
FCR (g fed/g gain)	0.86	1.03	1.11	1.25
Nitrogen retention (%)	40.0	29.9	31.0	25.9
Phosphorus retention (%)	22.5	29.5	39.1	43.7

Table 4.12 *Influence of high inclusion levels of extruded L. albus kernel meals fed to rainbow trout. Data derived from Burel et al. (1998).*

	Reference	50% Lupin	70% Lupin	70% + Attractan
Diet ingredients (g/kg)				
L. albus kernel meal	-	500	700	700
Fishmeal	480	230	120	125
Pea meal	200	60	28	13
Pregelled starch	150	60	-	-
Fish oil	110	90	90	90
L-Methionine	-	-	2	2
Attractant	-	-	-	10
Remains (uniform across treatments)	60	60	60	60
Diet proximate specifications				
Dry matter (g/kg)	866	863	902	877
Crude protein (g/kg DM)	444	431	449	456
Crude fat (g/kg DM)	170	175	187	185
Gross phosphorus (g/kg DM)	14.4	10.3	8.7	8.5
Gross ash (g/kg DM)	101	69	61	62
Gross energy (MJ/kg DM)	21.9	22.3	22.8	22.6
Fish performance criteria				
Initial weight (g)	27.0	27.0	27.0	27.0
Final weight (g)	79.0	80.0	87.7	84.0
DGC (%/d)	2.2	2.3	2.5	2.4
Feed intake (g/d/fish)	0.72	0.87	1.02	1.02
FCR (g fed/ g gain)	0.87	1.00	1.03	1.11
Nitrogen retention (%)	41.3	37.5	35.8	32.8
Phosphorus retention (%)	22.5	29.5	39.1	43.7

An additional aspect to the studies conducted by Burel et al. (1998) was the examination of plasma levels of the thyroxine (T_4) and tri-iodothyronine (T_3). From the first of the studies a negative correlation ($r^2 = 0.85$) was observed between the levels of T_4 and the level of *L. albus* kernel meal inclusion, suggesting the presence of goitrogenic compounds. The influence of *L. albus* kernel meal inclusion on T_4 though did not extend to T_3, with deiodonase activities demonstrated to have been sufficient to maintain adequate T_3 levels. In the later studies however, this same effect was not as evident, with variable levels of T_4 observed and growth not directly responsive to the inclusion level of *L. albus* kernel meal. It was subsequently proposed that these differences were a result of different crop characteristics, with the first study suspected to have had problems with low *L. albus* kernel meal iodine levels. These were unfortunately not able to be verified.

4.1.2 Atlantic salmon (Salmo salar)

Carter (1999) undertook a series of studies evaluating the potential of some plant meals to replace fishmeal in diets for the Atlantic salmon as part of Australia's Fisheries Research and Development Corporations Fishmeal Replacement Subprogram. Carter adopted a slightly different approach to addressing the use of plant proteins than that undertaken by other researchers in the Fishmeal Replacement Subprogram. The studies from Carter's project reviewed here examine primarily the use of supplemental enzymes, protein concentrates and the development of *in vitro* assays.

Carter et al. (1999) developed a series of *in vitro* assays to determine the protein/nitrogen digestibilities of a range of ingredients. These *in vitro* assays focussed on the use of either a commercial enzyme preparation or an enzyme preparation derived from Atlantic salmon pyloric caeci. A further assay method based on the measurement of a pH change was also investigated. Some of the ingredients were also evaluated under *in vivo* conditions, though the only ingredient of relevance to this review was the defatted soybean meal.

Several assessments of four plant protein resources; *L. angustifolius* whole-seed and kernel meals and soybean full-fat and defatted meals, were made using these three assay systems (Table 4.13). The commercial enzyme preparation consistently gave the highest digestibility values, where as the salmon enzyme preparation consistently gave the lowest values. The commercial enzyme preparation also had a slightly stronger correlation with the *in vivo* digestibilities than the other assays used in this study.

Based on the results of the commercial enzyme assay, the protein digestibility of the defatted soybean meal was slightly higher than that of the whole-seed *L. angustifolius*. Notably both the full-fat soybean meal and the *L. angustifolius* kernel meal had lower digestibility values again. The implications of these findings were not discussed.

From the salmon enzyme preparation assay, most digestibility values were less than 60%, with the exception of that of the *L. angustifolius* kernel meal which had a digestibility value of 80.2% Notably, the

digestibility of the defatted soybean meal was the lowest at 48.5% contrasting the results from the commercial enzyme preparation assay (Table 4.13).

The multi-enzyme pH change assay gave results in a similar pattern to the salmon enzyme preparation assay, though the level of digestibility was generally higher. With this assay the highest digestibility was observed with the *L. angustifolius* kernel meal (85.7%), similar to that of the salmon enzyme preparation assay. Protein digestibilities of the *L. angustifolius* whole-seed and full-fat soybean meals were, as with the salmon enzyme preparation assay, very similar. The defatted soybean meal again had the lowest protein digestibility (75.4%).

On average, across all assay methods employed in this study, the lupin meals had higher protein digestibility values than both the soybean and pea meals evaluated (Table 4.13). This higher digestibility of lupin protein is consistent with other studies on other species of salmonids (Hughes, 1988; Burel et al., 1998).

Despite the potential cost-efficiency of progressing such *in vitro* assays, the considerable variation depending on the assay system used still supports that the use of *in vivo* assays is paramount. In this regard, further evaluation of the nutritional value of lupin meals in this species, particularly with reference to soybean meals is clearly needed.

Table 4.13 In vitro digestible value of L. angustifolius and soybean meals fed to Atlantic salmon. Data derived from Carter et al. (1999).

	L. angustifolius		Soybean	
	Whole-seed	Kernel meal	Full-fat	Defatted
Ingredient Proximate Composition				
Dry matter (g/kg)	-	941	-	942
Crude protein (g/kg DM)	-	424	-	496
Apparent Protein Digestibility				
Commercial preparation - (%)	91.0	87.3	88.3	93.3
Salmon preparation - (%)	58.2	80.2	57.4	48.5
Multi-enzyme pH change assay - (%)	81.4	85.7	79.2	75.4
In vivo assay – (%)	-	-	-	92.8

In a study undertaken by Carter and Hauler (1999), the biological value of *L. angustifolius* (cv. Gungurru) kernel meal was compared against that of soybean meal and pea protein concentrate when fed to atlantic salmon. Each ingredient was included in the diet to constitute 40% of the total dietary protein. Digestibility of each of the diets was measured following the collection of faeces using the settlement collection method of Cho et al. (1982) after being fed the diets for at least four days.

From this study the highest apparent nitrogen digestibilities were those observed from the diets in which pea meal (93.8%) constituted 40% of the dietary protein. Second highest nitrogen apparent digestibility was of the diet in which soybean (92.0%) constituted 40% of the dietary protein. It should be noted though that the nitrogen apparent digestibility of *L. angustifolius* kernel meal (91.3%) was not significantly lower than either pea meal or soybean. Apparent energy digestibilties were highest from salmon fed the soybean diet (86.4%). Second highest was the pea meal diet (83.1%) in which the pea meal constituted 40% of the dietary protein. While the diet containing *L. angustifolius* kernel meal had the lowest apparent energy digestibility (80.5%). This was consistent with the lower protein and fat levels of this protein resource and the higher levels of indigestible non-starch polysaccharides. Of particular note though were the phosphorus digestibilities. Highest in this regard was the diet containing the *L. angustifolius* kernel meal (46.7%), next highest was the diet containing the pea meal protein (41.6%). The diet containing soybean meal (27.5%) had considerably poorer phosphorus digestibility than the *L. angustifolius* kernel meal, pea meal and even the reference diet (39.8%).

In this study, paired treatments, in which the supplemental enzyme phytase was added, were also examined. In both the soybean meal (93.4%) and the *L. angustifolius* diet (94.0%) treatments with added phytase, significant improvements in the nitrogen apparent digestibility were observed over those without the supplemental enzyme. Similar observations were not observed with the pea meal (94.1%). Most notable though was the influence on the apparent digestibility of dietary phosphorus. The addition of supplemental phytase improved the phosphorus apparent digestibility in all cases, with the highest phosphorus apparent digestibility observed in the pea meal diet with added phytase (57.2%). This was not significantly higher than that of the lupin meal diet with added phytase though (55.9%) or soybean meal diet (49.7%) with added phytase. In each case, the addition of phytase significantly improved phosphorus apparent digestibility of the diet, compared to that of the unsupplemented diet.

Carter and Hauler (1999) also examined the biological value of inclusion of the soybean meal, pea meal and *L. angustifolius* kernel meals in the diets. Notable were the higher nitrogen retention values of fish fed the *L. angustifolius* kernel meal diets (52.7%), which were significantly higher than both that of the soybean (44.2%) and pea seed meal (46.2%) diets.

Table 4.14 *Utilisation of soybean, L. angustifolius kernel meal and pea meal by Atlantic salmon (Salmo salar). Data derived from Carter and Hauler (1999).*

	Fishmeal	*Soybean*	*Lupin*	*Lupin+ phytase*	*Pea*
Diet ingredients					
Fishmeal	615.0	369.0	369.0	369.0	369.0
Soybean meal	-	357.0	-	-	-
L. angustifolius kernel meal	-	-	424.0	424.0	-
Pea meal	-	-	-	-	449.0
DL-methionine	-	8.5	10.0	10.0	4.0
Fish oil	138.0	154.8	154.8	154.8	154.8
Bentonite	123.3	87.5	19.0	19.9	-
Phytase (IU/kg)	-	-	-	1000.0	-
Remains (uniform across treatments)	23.2	23.2	23.2	22.3	23.2
Diet proximate specifications					
Dry matter (g/kg)	940	950	947	945	935
Crude protein (g/kg DM)	429	441	431	433	447
Crude fat (g/kg DM)	217	216	236	236	221
Gross ash (g/kg DM)	229.7	180	109.9	110.7	100.3
Gross energy (MJ/kg DM)	19.03	20.14	21.77	21.71	21.69
Fish performance criteria					
Initial weight (g)	34.7	34.7	34.8	35.2	34.8
Final weight (g)	78.9	75.9	78.6	76.2	75.1
DGC (%/d)	1.63	1.54	1.62	1.53	1.52
FCR (g fed / g gain)	1.7	1.7	1.4	1.4	1.6
Feed intake (g / fish / d)	1.11	1.07	0.96	0.87	0.99
Nitrogen retention (%)	44.5	44.2	52.7	55.8	46.2
Apparent Protein Digestibility (%)	90.5	92.0	91.3	94.0	93.8
Apparent Phosphorus Digestibility (%)	39.8	27.5	46.7	55.9	41.6
Apparent Energy Digestibility (%)	91.9	86.4	80.5	81.9	83.1

Carter and Hauler (2000) also evaluated the nutritional value of diets containing a *L. angustifolius* (cv. Gungurru) protein concentrate and compared the nutritional value of this processed form of the grain relative to that of defatted soybean meal and a pea protein concentrate. The three protein resources were included in diets at either 25% or 33% replacement of the fishmeal protein content of the diet (Table 4.15). Apparent digestibility of each of the diets was measured following the collection of faeces using the settlement collection method of Cho et al. (1982) after being fed the diets for at least four days.

The highest apparent nitrogen digestibilities were those observed from the diets in which the *L. angustifolius* protein concentrate replaced 33% of the fishmeal (Table 4.15). Second highest was the diet in which soybean replaced 33% of the fishmeal. Pea protein concentrate had the least influence on the nitrogen digestibility of the feeds. Apparent energy digestibilities were also highest with the diet in which the *L. angustifolius* protein concentrate replaced 33% and 25% of the fishmeal respectively. As with the apparent nitrogen digestibility, the pea protein concentrate also had the least influence on the apparent energy digestibility of the feeds.

Assessment of the biological value of the diets fed to the juvenile atlantic salmon in Carter and Hauler's (2000) study supported that each diet maintained growth equal to that of the control/reference diet (Table 4.15). Food consumption was significantly higher by fish fed the diet in which 33% of the fishmeal was replaced by *L. angustifolius* protein concentrate. 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 33% replacement *L. angustifolius* protein concentrate treatment also had a significantly poorer nitrogen retention, at only 30.2%, compared with 41.2% and 45.1% by fish when fed the soybean meal and pea protein concentrate respectively. Interestingly, both the soybean meal and pea protein concentrate treatments also had nitrogen retention values slightly higher than that of the fishmeal control, though only significantly so for the pea protein concentrate treatment at 33% replacement of the fishmeal.

The poorer utilisation characteristics of the *L. angustifolius* protein concentrate observed in this study contrasted those from earlier work by this group (Carter and Hauler, 1999), These differences were not fully explained, though it was suggested that the comparatively higher levels of non-starch polysaccharides in the *L. angustifolius* protein concentrate may have had an influence on its nutritional value. However, if this was the case then it could also be reasoned that addition of cellulose to some of the test diets should have had a similar effect (Table 4.15). Furthermore, if the levels of NSP were the key reason for the relative deterioration in biological value of the diet, then this should have been more apparent in the earlier study by this group (Carter and Hauler, 1999) where a kernel meal, with higher NSP levels than the concentrate was used. Notably, in this earlier work, the reverse was observed, with greater biological value being attributed to the *L. angustifolius* kernel meal than that of either the soybean or pea protein resources. Also of note from this earlier study

was the observation that fish fed diets containing *L. angustifolius* kernel meal had growth and feed conversion parameters equal to that of both the soybean and pea seed meals. Also in contrast were the influences of the *L. angustifolius* kernel meal on feed intake, with the protein concentrate inducing greater feed intakes at its higher inclusion level. The disparities between the results obtained with the kernel meal in the earlier study and the protein concentrate in the later study question the value of the concentration process to this commodity when fed to atlantic salmon, or raise questions on variability in grain quality attributes.

Based on the results from this study, Carter and Hauler (2000) supported that extruded Atlantic salmon feeds could reasonably contain in excess of 220 g/kg of *L. angustifolius* protein concentrate and 270 g/kg 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.

Though not studying lupins per se, a notable study by Arnesen et al. (1989) found that ethanol-soluble carbohydrates present in soybean meals had a significant influence on the utilisation of dietary nutrients by Atlantic salmon. This work is notable in the context of lupins in that it presents one of the few accounts that examines the specific effects to fish of ethanol-soluble carbohydrates, which are analogous to oligosaccharides, on the nutritive value of a plant protein resource. This study also examined the influence of ethanol-soluble carbohydrates on rainbow trout, but found no significant effects on nutrient utilisation, other than a slightly increased level of faecal matter output. The contrasting difference between rainbow trout and atlantic salmon was not fully explained and it is unclear as to whether this is a species difference or a factor attributable to either the freshwater (rainbow trout) or seawater (atlantic salmon) mediums used for the study of each species. However, despite no direct evidence to suggest so, it is likely that similar such influences of ethanol-soluble carbohydrates (oligosaccharides) from lupins may also have an influence on the utilisation of dietary nutrients by atlantic salmon and rainbow trout, particularly at high inclusion levels. This study highlights the need to examine the specific nutritional constraints to the use of these protein resources in the diets of salmonids.

Figure 13. Atlantic salmon, rainbow trout and lupin seeds, kernels, meals and diets.

Table 4.15 *Utilisation of soybean, L. angustifolius protein concentrate and pea protein concentrate by Atlantic salmon (Salmo salar). Data derived from Carter and Hauler (2000).*

	Control	*S25*	*S33*	*LC25*	*LC33*	*PC25*	*PC33*
Diet ingredients							
Fishmeal	601	451	400	451	400	451	400
Soybean meal	-	204	273	-	-	-	-
L. angustifolius protein concentrate	-	-	-	218	292	-	-
Pea protein concentrate	-	-	-	-	-	206	276
DL-methionine	-	3	5	4	6	5	6
Fish oil	155	160	159	157	156	167	169
Bentonite	48	-	-	-	-	-	-
Cellulose	50	36	17	24	-	43	27
Remains (uniform across treatments)	146	146	146	146	146	128	122
Diet proximate specifications							
Dry matter (g/kg)	941	948	943	925	910	927	933
Crude protein (g/kg DM)	419	413	413	425	425	413	406
Crude fat (g/kg DM)	263	258	268	272	260	260	258
Gross ash (g/kg DM)	130	80	80	80	70	70	60
Gross energy (MJ/kg DM)	21.86	22.66	22.94	22.81	22.65	22.76	22.90
Fish performance criteria							
Initial weight (g)	46.7	46.4	46.8	46.3	46.4	46.8	46.6
Final weight (g)	113.1	120.7	116.9	114.0	113.9	123.4	118.4
DGC (%/d)	1.96	2.14	2.04	2.00	1.99	2.18	2.08
FCR (g fed / g gain)	1.10	0.96	1.02	1.03	1.28	0.99	0.99
Feed intake (mg DM / g fish / d)	14.6	13.6	13.8	13.8	17.1	14.1	13.6
Nitrogen retention (%)	38.0	41.5	41.2	38.9	30.2	40.9	45.1
Apparent Protein Digestibility (%)	92.7	95.3	95.9	95.6	95.9	95.2	95.5
Apparent Energy Digestibility (%)	87.9	89.0	89.7	91.3	91.8	88.8	89.2

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

4.2 Seabreams

Perhaps the second best studied of the aquacultured fish that have been fed lupins, are the seabreams (including both *Sparus aurata* and *Pagrus auratus*). The results from the studies reviewed, in which lupins were fed to seabream, shows that lupin meals and particularly lupin kernel meals, are a valuable potential feed ingredient to these animals.

4.2.1 Gilthead seabream

Kissil and Lupatsch (2000) made an assessment of the apparent digestibilities of protein and energy of *L. angustifolius* (cv. Warrah) kernel meal and also a specially bred line of *L. angustifolius* (cv. Warrah) kernel meal (WT) with higher levels of methionine. Fishmeal was also evaluated for reference basis. Diets were prepared and fed to 300 g to 400 g gilthead seabream (*Sparus auratus*). Faeces were collected using stripping techniques.

These workers found that protein from both sources was highly available (>91%). Energy digestibilities of the kernel meals differed considerably, with the WT *L. angustifolius* kernel meal (61.7%) showing an energy digestibility substantially higher than that of the Warrah kernel meal (53.6%) (Table 4.16).

Table 4.16 Digestibility of a cultivar of L. angustifolius kernel meal and new experimental line, high in methionine, when fed to the gilthead seabream. Data from Kissil and Lupatsch (2000).

	L. angustifolius kernel meal		Fishmeal
	cv. Warrah	line WT	
Ingredient proximate specifications			
Dry matter (g/kg)	948	942	930
Crude protein (g/kg DM)	367	431	652
Crude fat (g/kg DM)	78	99	112
Ash (g/kg DM)	37	36	200
Nitrogen-free extractives (g/kg DM)	247	254	0
Methionine (g/kg DM)	2.5	5.1	19.5
Gross energy (MJ/kg DM)	18	19	20
Nutrient Apparent Digestibility Coefficients			
Protein (%)	91.9	93.9	82.1
Energy (%)	53.6	61.7	80.4

The earliest of the studies examining the value of lupins to a seabream species was that by Robaina et al. (1995). Though not studying specifically the nutritional value of the ingredients per se, these workers reported the digestible protein and lipid values of diets fed to juvenile (72 g initial weight) sea bream (*Sparus auratus*). The diets in the study contained incremented levels (10%, 20% or 30%) of either soybean meal or *L. angustifolius* whole-seed meal (cultivar used not detailed) (Table 4.17). Proximate specifications for either ingredient were not detailed. However, based on the comparative levels of oils added to the soybean test diets, it is likely that defatted-soybean meal was used. Prior to incorporation of the grain meals, these workers also examined the solubility and trypsin inhibitor activity of the soybean meal to ensure it had been heat-treated and soaked the lupin seeds in water for 24 h in an attempt to remove excess alkaloids.

Assessment of the nutritional value of the diets was made on a comparative basis only, as diets were not designed to directly determine the discrete nutritional value of the ingredients. The diets containing 30% of either meal had apparent protein digestibilities of 87.6% and 93.0% for soybean meal and *L. angustifolius* whole-seed meal respectively (it was suggested that this would approximate to a difference in ingredient apparent protein digestibilities close to 10% in favour of the *L. angustifolius* whole-seed meal).

As part of the same study, Robaina et al. (1995) examined the trypsin activity of fish from each of the treatments and interestingly observed higher levels of trypsin activity from fish fed the soybean meal diets than those fed the *L. angustifolius* whole-seed meal. It was also noted that a trend was consistent with the inclusion level of the *L. angustifolius* whole-seed meal and the trypsin activity. Furthermore, these workers also commented on the observation that the trypsin activity levels were not consistent with the levels of *in vivo* digestion determined for each of the diets.

These workers (Robaina et al., 1995) also studied the comparative biological value of both *L. angustifolius* whole-seed meal and soybean meal when fed to the sea bream (Table 4.17). As detailed earlier, the diets were formulated to contain incremented levels (10%, 20% or 30% of the diet) of either the soybean or *L. angustifolius* whole-seed meals. Diets were also formulated to approximately iso-nitrogenous and iso-lipidic on an as-fed basis. The diets were fed four times daily to juvenile (~ 40 g mean initial weight) sea bream for a ten week period.

After ten weeks, sea bream fed the 10% soybean meal diet had gained the most weight, being significantly greater than all other treatments excepting the 30% *L. angustifolius* whole-seed meal diet and the 20% soybean meal diet (Table 4.17). When growth performance was examined as a daily growth coefficient, a trend was observed in both groups of treatments, with decreasing growth performance seen with increasing levels of inclusion of either protein resource. Notably though, performance of fish fed corresponding diets of 10% lupin or soybean meal, 20% lupin or soybean meal or 30% lupin or soybean meal were not significantly different, suggesting similar biological value of both meals.

Table 4.17 *Utilisation of soybean and L. angustifolius whole-seed meals by the gilthead sea bream (Sparus auratus). Data derived from Robaina et al. (1995).*

	Control	***S10***	***S20***	***S30***	***L10***	***L20***	***L30***
Diet ingredients							
Fishmeal	766	690	613	536	690	613	536
Soybean meal	-	101	202	302	-	-	-
L. angustifolius whole seed meal	-	-	-	-	115	231	346
Fish oil	60	66	72	78	41	23	4
EPA 42 (enriched fish oils)	-	-	-	-	9	17	25
Cellulose	91	60	30	-	62	33	4
Remains (uniform across treatments)	83	83	83	83	83	83	83
Diet proximate specifications							
Dry matter (g/kg)	940	941	913	906	937	922	898
Crude protein (g/kg DM)	593	607	606	612	601	606	589
Crude fat (g/kg DM)	141	135	145	152	134	128	127
Gross ash (g/kg DM)	159	146	131	134	128	130	127
Gross energy (MJ/kg DM)	19.28	20.13	20.22	18.70	17.97	18.19	20.12
Fish performance criteria							
Initial weight (g)	38.3	40.3	39.4	37.0	38.5	38.7	39.6
Final weight (g)	60.1	64.5	62.0	56.5	60.3	59.1	61.5
DGC (%/d)	0.91	0.97	0.93	0.84	0.91	0.85	0.90
FCR (g fed / g gain)	1.64	1.59	1.64	1.82	1.59	1.89	1.79
Nitrogen retention (%)	24.9	22.4	19.7	21.9	19.0	24.9	27.7
Apparent Protein Digestibility (%)	92.9	93.6	86.2	87.4	95.5	94.5	93.0
Apparent Lipid Digestibility (%)	92.6	93.2	95.9	97.5	97.2	93.9	95.3
Trypsin activity (mUnits/mg protein)	0.12	0.09	0.15	0.02	0.04	0.01	0.01

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

Histological examination of the influence of each of the diets on glycogen and lipid deposition showed that fish fed the soybean meal based diets had an increased amount of lipid droplets around their pancreatic tissue in the liver. Eccentrically located cell nuclei were also observed in the hepatocytes from fish fed diets containing 20% or 30% of soybean meal. High levels of hepatocyte vacuolisation and disorganisation were observed from fish fed the diet containing 30% soybean meal. It was suggested that this may have been a symptom of phosphorus deficiency, with the level of diet phosphorus availability decreasing with increasing levels of soybean meal. Soybean phosphorus is predominant in the form of phytate and probably, unavailable to the fish. Minor histological differences were also observed between fish fed the control diet and the diets containing the *L. angustifolius* whole-seed meal. Some small increases in liver lipid droplets and some reduction in the amount of glycogen deposits were observed in fish fed diets containing the higher inclusion levels of *L. angustifolius* whole-seed meal. However, in comparison to the effects seen with the inclusion of soybean meal, those from fish fed the *L. angustifolius* whole-seed meal diets were considered minor.

Robaina et al. (1995) also examined the nitrogen excretion characteristics of fish fed each of the diets. Most notable were the differences between the ammonia excretion characteristics of fish fed the fishmeal based diet and all of the test diets containing the two plant protein resources and their respective incremental inclusion levels. Ammonia excretion peaked at 6 h post-feeding with fish fed the plant-protein meal diets, and at 4 h post-feeding with the control fishmeal based diet. Total ammonia excretion was also lower from fish fed the control fishmeal based diet. A significant negative correlation was also observed between the amount of ammonia excreted and the protein digestibility in each of the diets used in this study. These observations are consistent with an increased level of protein metabolism for energy derivation from the plant-protein meal diets.

One further notable observation was the trend of an increasing rate of nitrogen retention with increasing inclusion level of lupin in the diet (Table 4.17). This observation is also in accordance with the higher nitrogen/protein digestibility of these diets.

These workers advocated the high value of *L. angustifolius* whole-seed meal as an ingredient for sea bream, based on the good acceptance and high protein digestibilities of the protein resource, and considered the ingredient at least equal to, if not superior to soybean meal.

An *in vitro* examination of a range of protein resources was also undertaken by Alarcon et al. (1999) who studied the influence of endogenous protease inhibition by the various protein resources. In this study, the stomach and pyloric caeci of juvenile sea bream (*Sparus auratus*) were used to produce enzyme extracts for *in vitro* evaluation of each of the protein resources. Samples were incubated at 25°C for fixed time intervals with a standardised amount of protease. At the end of the time interval the reaction was stopped and the remaining protein measured. The first experiment by these workers identified

protease inhibition activity on a basis relative to a control protein source of casein. Using this method lupin meal (CP: 429 g/kg DM; though species, cultivar and processing form not stated – assumed to be *L. albus* whole-seed meal) was found to induce 42.9 ± 2.6% inhibition compared to casein (assumed to be 0% inhibition). In comparison, solvent extracted soybean meal produced protease inhibition of 39.9 ± 3.0% and raw soybean meal 42.6 ± 6.7%. Inhibition for horse bean meal and green pea meal were reported as 49.1 ± 2.6% and 53.3 ± 1.9% respectively. Lowest levels of protease inhibition observed in the experiment were those of bloodmeal (1.0 ± 2.0%). No reasoning was offered for the relative ranking of the protease inhibition by each of the protein resources, other than as endogenous inhibitors present in the protein meals. A reduction of protease inhibition through grain processing was noted between the raw and solvent extracted soybean meals. The higher level of protease inhibition by the lupin meal is intriguing given that trypsin inhibitor activities (TIA) and chymotrypsin inhibitor activities (CTIA) of raw soybean were well established as being considerably higher than that of any of the lupin meals (White et al., 2000). The levels of TIA reported in pea seed meal are also lower than that of raw soybean (Petterson et al., 1997) yet this ingredient too reputedly induced greater protease inhibition than the soybean meals in this study.

A second experiment by these workers (Alarcon et al., 1999) examined the reaction rate kinetics of the protease reactions in the presence of each of the inhibitors, again relative to the control casein. This experiment also supported the initial study of the relative assessment of protease inhibition by each protein resource, but also added information of the influence of each protein resource on the reaction rate kinetics. While the determination of such rate curves has some value, the reporting of the Michaelis-Menten constant (K_m) for the reaction curves associated with each protein resource and the maximal reaction rate in the presence of each substrate (V_{max}), would have provided considerably more value to the study and allowed critical assessment of the nature of the inhibition process associated with each of the protein resources (Rawn, 1989).

In a further experiment, the influence of acidification of the incubation media was examined to mimic the digestive environment of the stomach. Protease inhibition of the lupin meal was significantly reduced from 47.5% to 43.0% with acidification. However, despite this slight improvement in the protease activity in the presence of lupin meal, it was still considerably higher than that of the solvent extracted soybean meal (32.4% and 33.0% for control and acidified media treatments respectively, Alarcon et al., 1999). Again these results were contrary to the performance seen *in vivo* (Robaina et al., 1995; Kissil and Lupatsch, 2000). What this study did highlight though is a key need for linkage of both *in vitro* and *in vivo* assessment data.

A study by Kissil and Lupatsch (2000) evaluated the use of two lines of *L. angustifolius* (cv. Warrah) kernel meal, when fed to the gilthead seabream (*Sparus aurata*). One of these lines had twice the methionine content of that usually found in *L. angustifolius* kernel meal (2.5 g/kg DM cf. 5.0 g/kg DM).

The high methionine line of *L. angustifolius* kernel meal was the result of the transgenic introduction of a sunflower protein gene being expressed within the lupin seed (Molvig et al., 1997). The study was designed primarily to determine the value and potential of this methionine enriched line of *L. angustifolius* (cv. Warrah). Diets were formulated to contain equal quantities of *L. angustifolius* kernel meal and fishmeal. Additional diets were fortified with crystalline DL-methionine to balance total dietary methionine content between treatments. A reference diet based on fishmeal was also included. All diets were iso-energetic and iso-nitrogenous on a digestible basis (Table 4.18).

Growth by gilthead seabream fed the diets supported that both lines of *L. angustifolius* kernel meal had considerable potential to be used as a protein meal replacing fishmeal in the diets of this species. However, the addition of DL-methionine or the use of the methionine enriched line of *L. angustifolius* kernel meal provided no apparent additional benefit (Table 4.18). Feed intakes of fish fed these diets were also comparable to those observed of fish fed the reference diet. The observation of these consistently comparable feed intakes was a noted benefit of the *L. angustifolius* kernel meal diets in reflect to earlier experiences of the workers with canola and soybean protein concentrate studies. The nitrogen and energy retention values observed from fish fed the diets showed significantly lower nitrogen retention with the *L. angustifolius* kernel meal diets, but slightly higher energy retention.

That no differences were observed between the *L. angustifolius* kernel meal diets and the fishmeal based reference provides good account of the viability of *L. angustifolius* kernel meals in diets for this species. However, that no differences were observed between the *L. angustifolius* kernel meal diets and those fortified with DL-methionine also indicates that it is unlikely that this amino acid was limiting growth in this instance. For specific value of the additional methionine to be determined, either in the diets or the *L. angustifolius* kernel meals the dietary methionine must be made to be the first limiting nutrient in the diets being examined. It would be of value to determine the responses of gilthead seabream to diets formulated to contain slightly less than the optimal level of digestible protein whilst optimising dietary digestible energy intake, thereby enforcing potential amino acid limitations on the growth of these fish.

Figure 14. Red seabream with L. angustifolius seeds, kernels and meal

Table 4.18 *Substitution of L. angustifolius kernel meals for fishmeal in diets for the gilthead sea bream (Sparus auratus). Data derived from Kissil and Lupatsch, 2000.*

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Diet ingredients					
Fishmeal	700	445	445	445	445
L. angustifolius kernel meal (cv. Warrah)	-	445	445	-	-
L. angustifolius kernel meal (ln. TG)	-	-	-	445	445
DL-methionine	-	-	1.5	-	3.0
Wheat meal	190	-	-	-	-
Remains (uniform across treatments)	110	110	110	110	110
Diet proximate specifications					
Dry matter (g/kg)	900	893	911	909	909
Crude protein (g/kg DM)	502	502	495	491	459
Crude fat (g/kg DM)	189	197	196	205	186
Nitrogen-free extractives (g/kg DM)	123	137	141	122	115
Gross ash (g/kg DM)	142	110	104	105	95
Gross energy (MJ/kg DM)	22.28	23.29	22.54	22.79	21.24
Total methionine (g/kg DM)	14.6	10.2	11.6	11.2	13.1
Fish performance criteria					
Initial weight (g)	27.0	27.4	27.4	27.5	27.3
Final weight (g)	94.8	100.4	96.7	99.0	99.0
DGC (%/d)	2.60	2.72	2.63	2.68	2.69
Feed intake (g/fish/d)	1.11	1.21	1.23	1.23	1.22
FCR (g fed / g gain)	1.41	1.42	1.52	1.48	1.5
Nitrogen retention (%)	33.2	31.9	30.1	29.8	29.8
Energy retention (%)	44.0	48.4	47.1	45.0	44.7

4.2.2 Red seabream / Pink Snapper

Another species of the Sparidae family that has been used to study the nutritional and biological value of lupins is the red seabream (*Pagrus auratus*). The species has previously been reported using a number of synonyms; *Chrysophrys auratus* and *C. unicolour* in Australasia; and *Pagrus major* and *C. major* in Indo-China-Japan, and is commonly referred to as the pink snapper in Australiasia (Paulin, 1990). Independent and reproductively isolated populations are recognised between Australasia and Japan, but are now interpreted as the same species, *Pagrus auratus* (Paulin, 1990). This species has been used for detailed examination of the potential for dietary lupin incorporation in Australia since 1994 (Jenkins et al., 1994).

Glencross et al. (2002) made an assessment of the apparent digestibilities of protein (as N x 6.25) and energy of *L. angustifolius* (cv. Warrah) kernel meal and also a specially bred line of *L. angustifolius* (cv. Warrah) kernel meal (WT) with higher levels of methionine. Additional ingredients evaluated included wheat gluten, and fishmeal (Table 4.19). All ingredients were evaluated using the diet substitution assessment method and faeces collected based on the faecal settlement method described by Allan et al. (1999).

Table 4.19 *Digestibility of a cultivar of L. angustifolius kernel meal and a new line, high in methionine, when fed to red seabream. Data from Glencross et al. (2002).*

	L. angustifolius kernel meal		Wheat gluten	Fishmeal
	cv. Warrah	*line* WT		
Ingredient proximate specifications				
Dry matter (g/kg)	883	897	910	908
Crude protein (g/kg DM)	465	484	838	716
Crude fat (g/kg DM)	55	74	9	96
Ash (g/kg DM)	37	37	8	151
Nitrogen-free extractives (g/kg DM)	452	405	145	38
Methionine (g/kg DM)	3.7	6.1	12.0	20.5
Gross energy (MJ/kg DM)	20.6	21.3	22.6	21.3
Nutrient Apparent Digestibility				
Protein	98.7	99.8	102.0	81.3
Energy	56.3	64.0	84.3	87.8

These workers found that protein from both *L. angustifolius* (cv. Warrah) lines was highly available (>98%), similar to that of wheat gluten (100%) and significantly higher than from fishmeal (87%). Energy digestibilities of these ingredients for *P. auratus* differed considerably, with the high-MET *L. angustifolius* kernel meal (64%) showing energy digestibilities significantly higher than that of the low-MET *L. angustifolius* kernel meal (56%). In comparison wheat gluten and fishmeal had energy digestibilities of 84% and 88% respectively (Table 4.19).

The higher energy digestibilities of the high methionine variety of lupin were attributed to the higher levels of both protein and lipid in this line of the *L. angustifolius* Warrah cultivar.

The earliest work examining the use of lupins in diets for red seabream is that of Jenkins et al. (1994). In this study whole-seed *L. angustifolius* (cv. Gungurru) meal was substituted into a diet at the expense of soybean meal. Diets were formulated to be approximately iso-nitrogenous and iso-energetic on a crude basis, though no formulations or proximate details of the diets were provided. Diets were fed to an equal approximated ration based on fish size.

Growth of fish in each of the treatments was essentially the same. Similarly the feed conversion ratios, though very high, were also not significantly different. What this trial suggests is that there was some capacity for whole-seed *L. angustifolius* meal to replace either part or all of the soybean component in the diets formulated for this trial. However, the data are of relatively limited value in that an objective assessment cannot be made of whether the diets were nutrient limiting, and therefore whether the growth responses can really be attributed to the changes to the diet.

Table 4.20 *Substitution of L. angustifolius whole-seed meal for soybean meal in the diet for the red sea bream (Pagrus auratus). Data derived from Jenkins et al. (1994).*

	Diet 1	Diet 2	Diet 3
Ingredients			
L. angustifolius whole-seed meal	280	150	-
Soybean meal (defatted)	-	130	200
Fish performance criteria			
Initial weight (g)	77.7	77.7	77.6
Final weight (g)	140.8	143.1	141.7
DGC (%/d)	1.67	1.72	1.69
FCR (g fed / g gain)	1.76	1.73	1.81

Subsequent work undertaken by Petterson et al. (1995; 1997; 2000 and unpublished) evaluated the biological value of a range of lupin species and cultivars when fed to the red seabream. Using primarily growth trials as a means of performance evaluation, these workers have examined a range of issues from inclusion rates to processing variables.

One of these reported studies (Petterson et al., 1999) based the diets for the red seabream on the reference diet developed in Australia for this species by Quartararo et al. (1992). In this study incremented amounts of whole-seed *L. angustifolius* (cv. Gungarru) meal were substituted into the reference diet at levels from 10% to 50%, at 10% increments (Table 4.21).

Growth and food conversion by fish fed the experimental diets was essentially uniform up to an inclusion level of 40% of whole-seed *L. angustifolius*. At 50% inclusion of whole-seed *L. angustifolius* both growth and food conversion deteriorated. Differences in feed intake were only notable at an inclusion level of 50% of whole-seed *L. angustifolius*, suggesting that the diets were still well accepted by the fish up to these higher inclusion levels.

Limiting to the full interpretation of the data from this study though, is either the balancing of protein and energy in all of the experimental diets and/or the inclusion of additional negative control treatment(s). Without these controls full assessment on the biological value of the inclusion of whole-seed *L. angustifolius* from this study cannot be made, other then to assess the influence on feed intake.

Table 4.21 *Substitution of L. angustifolius whole-seed meal into a reference diet for the red sea bream (Pagrus auratus). Data derived from Petterson et al. (1999). Formulations not provided.*

	Reference	*10%*	*20%*	*30%*	*40%*	*50%*
Diet proximate specifications						
Crude protein (g/kg DM)	490	457	443	424	412	388
Crude fat (g/kg DM)	103	108	114	117	123	119
Lysine (g/kg DM)	32	29	27	25	24	22
Methionine + Cystine (g/kg DM)	13	14	13	12	11	9
Fish performance criteria						
Initial weight (g)	30.4	30.4	29.9	30.0	29.8	30.5
Final weight (g)	78.3	78.6	77.7	77.6	76.9	73.9
DGC (%/d)	2.41	2.43	2.43	2.41	2.40	2.23
FCR (g fed / g gain)	1.24	1.23	1.24	1.25	1.26	1.37

A later study by Petterson et al., (Petterson, Jenkins and Evans, reviewed in Petterson, 2000) examined the variability of two different cultivars of *L. angustifolius* whole-seed meal (cv. Gungurru and cv. Warrah) and also compared these meals against kernel meals and a protein concentrate. The diets were generally formulated to be iso-nitrogenous and iso-energetic, with the exception of two treatments (Table 4.22). These diets were fed to red seabream to apparent satiety twice daily for a period of eight weeks.

The results of this study demonstrated that there was some variability between the nutritional value of the different cultivars of *L. angustifolius* (Table 4.22). While not readily apparent it is suggested that this difference may be attributable to differences in digestible protein and/or energy between the different cultivars. Evaluation of the kernel meal and protein concentrate suggested that when included in the diet on an iso-nitrogenous basis, then they were at least of equivalent nutritional value to that observed for the whole-seed meal.

Figure 15. Red seabream (Pagrus auratus) with (L to R) L. luteus seed, L. angustifolius kernels, L. angustifolius kernel meal and L. angustifolius seeds

Table 4.22 *Substitution of L. angustifolius whole-seed, kernel meals and protein concentrates into a reference diet for the red sea bream (P. auratus). Data derived from Petterson (2000) and unpublishded (Petterson et al., 1999).*

	Gungurru 300	Warrah 300	Kernel 235	Kernel 300	Concentrate 188
Ingredients					
L. angustifolius whole-seed meal (cv. Gungurru)	300	-	-	-	-
L. angustifolius whole-seed meal (cv. Warrah)	-	300	-	-	-
L. angustifolius kernel meal (cv. Gungurru)	-	-	235	300	-
L. angustifolius protein concentrate (cv. Gungurru)	-	-	-	-	188
Wheat	203	206	274	216	312
Di-calcium phosphate	10	7	9	9	9
Fish meal	420	420	415	410	420
Fish oil	42	42	42	40	46
Remains (uniform across treatments)	26.64	26.64	26.64	26.64	26.64
Diet proximate composition (calculated)					
Protein (g/kg DM)	437	444	446	464	453
Fat (g/kg DM)	109	113	109	110	112
Gross energy (g/kg DM)	18.7	20.6	20.2	20.2	20.4
Fish performance criteria					
Initial weight (g)	33.5	33.5	33.3	33.3	33.1
Final weight (g)	80.6	85.6	80.9	78.9	82.4
DGC (%/d)	1.99	2.15	2.01	1.95	2.07
FCR (g fed / g gain)	1.31	1.26	1.32	1.28	1.26

Table 4.23 *Evaluation of extrusion processing and L. luteus whole-seed meal inclusion on the performance of red seabream (P. auratus). Data from Petterson et al. (1998).*

	Reference 1	Reference 2	Diet 1	Diet 2	Diet 3
Diet processing method	Pressed	Extruded	Pressed	Extruded	Extruded
Ingredients					
L. angustifolius whole-seed meal	-	-	300	300	-
L. luteus whole-seed meal	-	-	-	-	23.5
Diet proximate specifications					
Crude protein (g/kg)	485	485	387	387	387
Crude fat (g/kg)	102	102	100	100	102
Methionine (g/kg)	14	14	11	11	11
Fish performance criteria					
Initial weight (g)	27.7	27.4	27.6	27.4	27.4
Final weight (g)	64.6	64.5	62.9	66.4	68.2
DGC (%/d)	1.79	1.81	1.74	1.88	1.95
FCR (g fed / g gain)	1.46	1.35	1.66	1.45	1.37

A further study by Petterson et al. (1998) examined the use of *L. luteus* whole-seed meal and also the influence of extrusion processing, over the use of pellet pressing (Table 4.23). In this study, the *L. angustifolius* whole-seed meal diet was based on the 30% inclusion diet that was previously identified as a reasonable inclusion level of this meal. The *L. luteus* whole-seed meal diet was formulated to the same crude protein level as the *L. angustifolius* whole-seed meal diets.

Growth and feed conversion by fish fed these diets demonstrated that extrusion processing of the feeds improved their utilisation by red seabream. This influence of processing was observed on both the reference and *L. angustifolius* whole-seed meal diets. This observation suggests that the improvement was not related to lupin inclusion per se, but probably some other common factor to both diets. It is likely that the extrusion processing may have promoted the gelatinisation of starch components of the wheat components included in the diet, and that this may be providing additional value to the diets.

The inclusion of *L. luteus* whole-seed meal in the diet at a level iso-nitrogenous with that of the *L. angustifolius* whole-seed meal diet, suggested that the protein of the *L. luteus* whole-seed meal has at least equal, if not slightly superior nutritional value to that of *L. angustifolius* whole-seed meal. Whether this facet of the nutritional value of *L. luteus* is related to its protein digestibility or just its total protein content is unknown. However, further research on *L. luteus* is certainly warranted.

4.3 Silver perch

As part of Australia's Fisheries Research and Development Corporation's Fishmeal Replacement Subprogram, Allan and Rowland (1998), studying the Australian native freshwater fish the silver perch (*Bidyanus bidyanus*), reported a highly comprehensive series of studies on the nutritive value of lupins to this species. In this compendium of work Allan et al. (1998a; b) examined in detail the nutritional value of *L. angustifolius* (cv. Gungurru) whole-seed and kernel meals and also that of *L. albus* (cultivar not defined) whole-seed and kernel meals.

Initial evaluation of the apparent digestibility values of whole-seed meals of both *L. angustifolius* and *L. albus*, in conjunction with a suite of other ingredients, identified that both of these lupin species have considerable nutritional potential (Allan et al., 1999). Other ingredients evaluated included a range of soybean meals and pea meal (Table 4.24).

This study demonstrated that the whole-seed meals of both *L. angustifolius* and *L. albus* had substantially lower dry matter and energy apparent digestibilities than that of the soybean meals, but that they had equivalent protein digestibility. In comparison to the pea meal, *L. albus* whole-seed meal had equivalent or superior nutritional attributes and *L. angustifolius* whole-seed meal had inferior dry matter and energy apparent digestibilities, but superior apparent protein digestibility. The apparent digestibilities of the two key amino acids; lysine and methionine differed slightly. Lysine apparent digestibility in both lupin species was similar to that observed from the range of soybean meals, though it was substantially better than that of the pea meal. The methionine apparent digestibility, critically important given the paucity of this amino acid in lupin meals, was marginally poorer in *L. albus* than the soybean meals, but the methionine apparent digestibility of the *L. angustifolius* whole-seed was even poorer still (Table 4.24).

These results contrast some of the findings from other species, such as salmonids and seabreams (see section 4.1 and 4.2), where the protein digestibility of the lupin whole-seed meals was substantially higher than that observed for soybean meals. It is suspected that the differences may be accounted for by the different trophic status of the species in concern, with salmonids and seabreams being recognised carnivores and silver perch a recognised omnivore.

In a second, more comprehensive study on lupin nutritive value, both the whole-seed and kernel meals of both *L. angustifolius* and *L. albus* were evaluated (Allan et al., 1998a). Each ingredient was included in test diets at both 27.7% and 49.5% of the total diet. These inclusion levels were chosen to represent 30% and 50% inclusion of the ingredient in the diet respectively.

The apparent digestibility of protein (measured as nitrogen x 6.25) was not significantly influenced by the variety of lupin used in this study. Similarly, the inclusion level of lupin also, did not influence the apparent digestibility

of protein. The removal of the seed coat however, did significantly improve the apparent digestibility of protein of both varieties of lupins. Although the increase was significant, the magnitude of the improvement in apparent protein digestibility was less that 5% in both cases (Table 4.25). These observations were consistent with very high levels of apparent protein digestibility in both lupin species overall.

Both dry matter and energy apparent digestibilities were significantly affected by the species of lupin used, whether it was in kernel meal or whole-seed meal forms and also whether it was included at 30% or 50% in the diet (Table 4.25). It was suggested that these differences were a direct reflection in the changes of NSP between the whole-seed and kernel meals, between *L. angustifolius* and *L. albus* species, and that with the increasing inclusion level of each in the diet, that the NSP were having a compounded effect on the nutritive value of the diet.

The apparent digestibility of phosphorus differed significantly between the *L. angustifolius* and *L. albus*, meals and inclusion level, but was not affected by processing form (ie. kernel meal or whole-seed meal forms) (Table 4.25). This has considerable implications for development of phosphorus limiting diets, particularly so given the observations of Burel et al. (2000a), who reported the phosphorus digestibility of *L. albus* to be considerably superior to many other plant protein resources. In the study of Allan et al. (1998a) though, the data clearly shows the phosphorus utilisation from *L. angustifolius* to be significantly superior to that *L. albus* (Table 4.25).

Figure 16. Kernels of L. albus and L. angustifolius and L. albus kernel meal.

Table 4.24 *Digestibility of L. angustifolius and L. albus whole-seed meals, Pea meal and several varieties of soybean meals fed to the silver perch. Data derived from Allan et al. (1999).*

Nutrient	*L. angustifolius*	*L. albus*	Field pea (Dunn)	Soybean		
	whole-seed	whole-seed	whole-seed	solvent extr.	expeller extr.	full-fat
Ingredient Proximate Composition						
Protein (g/kg DM)	341	376	255	478	475	358
Fat (g/kg DM)	57	62	11	37	64	195
Ash (g/kg DM)	28	37	34	80	63	55
Nitrogen-free extractives (g/kg DM)	574	525	700	405	398	392
Gross energy (MJ/kg DM)	17.9	20.9	17.0	17.0	20.9	23.3
Lysine (g/kg DM)	14	15	17	33	30	23
Methionine (g/kg DM)	2	3	3	7	8	6
Nutrient Apparent Digestibility						
Dry matter	50.3	64.7	62.0	73.1	81.4	74.9
Nitrogen	96.6	96.1	83.3	95.3	97.2	92.1
Energy	59.4	72.7	67.0	81.5	85.2	78.7
Lysine	98.1	96.6	86.3	98.1	97.3	95.3
Methionine	83.9	92.2	87.5	96.4	97.6	95.5

Table 4.25 Digestibility of L. angustifolius and L. albus whole and kernel meals at fed at variable inclusion levels to the silver perch. Data derived from Allan et al. (1998a).

Nutrient	*L.angustifolius*				*L. albus*			
	Whole-seed 30%	Whole-seed 50%	Kernel 30%	Kernel 50%	Whole-seed 30%	Whole-seed 50%	Kernel 30%	Kernel 50%
Dry matter	50.3	50.8	67.6	68.9	64.7	59.4	77.8	68.2
Nitrogen	96.6	95.8	100.3	99.9	96.1	97.0	101.4	97.3
Energy	59.4	58.4	74.0	75.0	72.7	67.1	85.2	74.7
Phosphorus	71.8	72.0	80.1	78.0	77.5	67.0	73.8	61.0
Lysine	98.1	96.8	99.5	99.7	96.6	98.4	102.5	98.5
Methionine	83.9	95.5	91.7	96.4	92.2	94.5	97.3	97.3

Proximate compositions of the ingredients is provided in Table 4.24.

In contrast to other reported studies in which lupins have been evaluated in a fish species, the study of Allan et al. (1998a) is the only one to also evaluate the individual amino acid digestibilities. Of particular note are the digestibilities of the amino acids lysine and methionine, both because of their value to fish as an essential nutrient, and their limitation in plant meals relative to the requirement of most fish species (Kaushik, 1998b). Apparent digestibility of lysine was very high (>96% for all test cases). Significant differences in lysine digestibility were only apparent between the diets that included either whole-seed or kernel meals, with higher digestibilities observed from the kernel meals of both lupin species. The species of lupin or inclusion level used did not influence apparent lysine digestibility. In contrast to that observed for lysine, only the inclusion level of the lupin significantly influenced the apparent digestibility of methionine from lupin meals. This was only observed from the *L. angustifolius* meals, with the methionine apparent digestibilities from the *L. angustifolius* meals improving with the higher inclusion levels.

Allan et al. (1998a) also examined the levels and composition of NSP found in both the diets and faeces of silver perch fed the diets used in this study. The primary observation from this was that very little of any of the NSP classes were digested. These observations led Allan et al., (1998a) to claim that NSP are poorly digested by silver perch and as a consequence have little more value than fillers such as cellulose. These claims were justified based on the low levels of NSP digestion measured in this study and that no major changes in the composition of the NSP were observed between the diets and the faeces, supporting no selective absorption or digestion. Notably the level of dry matter digestion was also consistent with about 30% of the ingredient not being digested, similar the level of NSP in the lupin meals.

The results from this study show that distinct differences can exist between the nutritional value of plant protein resources, from plants even within the same genus. Similar to the observations in the study by Hughes (1991) with rainbow trout, the removal of the lupin seed coat to produce a kernel meal significantly improves most aspects of the nutritional value of both *L. angustifolius* and *L. albus* meals. Based on the observed nutritional value of both of *L. angustifolius* and *L. albus* meals, Allan et al. (1998a) suggested that both species would be suitable in diets for silver perch. However, because of slight deterioration in the nutritional value of *L. albus* at high inclusion levels that, *L. angustifolius* meals would probably be the better plant protein resource for use in silver perch diets.

Figure 17. Silver perch (Bidyanus bidyanus)

In another study by the same group of workers, Booth et al. (2001) examined the nutritional value of a range of other plant legumes, including field peas, faba beans, chick peas and vetch, but not any of the lupin species (Table 4.26). In this study each of the grains was also evaluated in whole-seed meal and kernel meal forms. Though no direct comparisons can be made from this study with the results from that of Allan et al. (1998a), generalisations on the relative values of the plant protein resources can be made. Notable between the two studies where the substantially higher level of apparent protein digestibility of the lupin meals. The only exception to this was the faba beans, which had a comparable level of apparent protein digestibility. Apparent energy digestibilities of each of the plant legumes in this study were comparable with that of both *L. angustifolius* and *L. albus* meals, though the lupin meals typically had slightly higher apparent energy digestibilities. Apparent dry matter digestibilities were also very comparable between each of the plant legume meals in the study by Booth et al. (2001) and both *L. angustifolius* and *L. albus* meals from the study by Allan et al. (1998a).

In a subsequent study, Allan et al. (1998b) evaluated the biological value of *L. angustifolius* (cv. Gungurru) kernel meal against dietary fillers of cellulose and diatomaceous earth in a summit-dilution study (Table 4.27). In this study, Allan et al. (1999b) included incremented amounts of *L. angustifolius* kernel meal (10%, 20%, 30%, 40%, 50%, 60%, 70% and 80% of the diet) with diatomaceous earth, also at various inclusion levels, being the appropriate negative control. The fish were fed on a pair-fed, restricted basis to remove feed intake variables as a confounding issue.

In this study Allan et al. (1998b) found that diets for silver perch could accommodate up to 600 g/kg of *L. angustifolius* kernel meal, without loss in biological performance, based on fish weight gain, protein deposition and nitrogen retention efficiency. It was however, observed that the efficiency of protein use began to decline when fish were fed diets with greater than 50% of *L. angustifolius* kernel meal. The decline in nitrogen retention efficiency when fish were fed diets containing 70% and 80% of *L. angustifolius* kernel meal, was not related to a decline in digestible protein or energy content of the diets. It was suggested that possible causes for this deterioration in performance were deficiencies in available amino acids and/or the presence of an anti-nutrient(s) that inhibited the dietary protein utilisation by this species. Subsequent analysis of the digestible amino acid content of the diets suggested that the requirements for dietary lysine were greater than that provided, though the total digestible sulfur amino acids were close to limiting. Retrospectively, it may have been valuable to have further detailed the assessment of the digestible methionine value specifically, as it is this amino acid that is usually first limiting in formulations with high *L. angustifolius* kernel meal contents (van Barneveld, 1999).

Table 4.26 Nutritional value of various plant legume meals to fed silver perch. Data derived from Booth et al. (2001)

	Ingredient Proximate Specifications (g/kg)				ngredient 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

* Fibre is acid detergent fibre (ADF).

Table 4.27 Performance characteristics of silver perch fed summit-diets evaluating L. angustifolius kernal meal. Data derived from Allan et al. (1998b).

	L. angustifolius kernel meal substitution									Diatomaceous earth substitution			
Parameter	Reference	10%	20%	30%	40%	50%	60%	70%	80%	10%	20%	30%	40%
Diet specifications (g/kg)													
Crude protein	369	341	360	351	364	376	386	385	427	331	n/s	n/s	215
Crude fat	50	49	51	55	57	59	57	47	50	40	n/s	n/s	24
Energy (MJ/kg)	19.5	18.9	19.1	19.3	19.3	19.2	19.7	19.5	19.8	16.1	n/s	n/s	11.3
Digestible protein (g/kg)	323	331	340	348	356	365	373	381	390	290	258	226	194
Digestible energy (MJ/kg)	14.5	14.6	14.7	14.7	14.8	14.9	15.0	15.0	15.1	13.1	11.6	10.2	8.7
Fish performance													
Initial weight (g)	1.82	1.82	1.82	1.82	1.82	1.82	1.82	1.82	1.82	n/s	n/s	n/s	n/s
Final weight (g)	16.72	16.62	18.02	16.72	15.22	15.62	15.02	13.02	12.52	n/s	n/s	n/s	n/s
DGC (%/d)	1.78	1.77	1.87	1.78	1.68	1.71	1.66	1.51	1.47	n/s	n/s	n/s	n/s
FCR (g fed / g gain)	1.5	1.6	1.5	1.6	1.6	1.7	1.7	1.8	1.9	n/s	n/s	n/s	n/s
Nitrogen retention (%)	26.2	28.7	29.7	27.2	24.4	24.7	24.1	22.1	19.2	n/s	n/s	n/s	n/s

All values are on an as-fed basis. n/s: not stated.

4.4 Asian seabass / Barramundi

The work of Williams (1998) presents another comprehensive evaluation of both the nutritional and the biological value of some key feed ingredients with potential for the Asian seabass, *Lates calcarifer*. Using both digestibility and summit-dilution studies the relative value of a range of protein meals, including *L. angustifolius* (cv. Gungarru) kernel and defatted and full-fat soybean meals was demonstrated. To date, the work reported by these researchers is the only account of lupin meals being fed to this species.

Evaluation of the digestibility of *L. angustifolius* (cv. Gungurru) kernel meal in *L. calcarifer*, using faecal stripping collection methods, supported that the nutritive value of this grain resource was similar to that observed of this grain in other carnivorous fish species (Table 4.28). Protein digestibility of the *L. angustifolius* kernel meal was very high at 98%. However, in accordance with the high levels of NSP in the kernel meal, the dry matter and energy digestibilities were considerably lower (McMeniman, 1998). The protein digestibility values of *L. angustifolius* kernel compared very favorably to that of defatted soybean meal which had dry matter, protein and energy digestibilties of 56%, 86% and 69% respectively (Table 4.28). Dry matter and energy digestibilities of the full-fat soybean meal were considerably higher than that of the defatted soybean meal, though the protein digestibility was essentially unchanged. The full-fat soybean meal had dry matter, protein and energy digestibilities of 69%, 85% and 76% respectively.

Table 4.28 *Digestibilities of L. angustifolius kernel and two types of soybean meals when fed to Asian seabass. Data derived from McMeniman (1998).*

	L. angustifolius	Soybean	
	Kernel meal	Defatted	Full-fat
Ingredient proximate composition			
Dry matter (g/kg)	895	910	910
Crude protein (g/kg DM)	441	529	448
Crude fat (g/kg DM)	88	16	183
Ash (g/kg DM)	27	72	53
NFE (g/kg DM)	444	383	316
Gross Energy (MJ/kg DM)	19.1	19.9	21.7
Nutrient Apparent Digestibility Coefficients			
Dry matter (%)	60.6	55.8	69.0
Protein (%)	98.1	86.0	84.8
Energy (%)	61.5	69.4	75.9

Williams (1998) undertook a series of studies to determine the biological value of several alternative protein resources including kernel meal of the lupin *L. angustifolius* (var. Gungurru). Using a summit-dilution approach the kernel meals were incorporated into a fish meal based summit diet at incremented levels from 100 g/kg to 700 g/kg of diet, at 100 g/kg increments. A series of control diets, where diatomaceous earth was added into the summit diets at 100, 200, 300 and 400 g/kg of diet were also included as treatments in the study (Table 4.29). A similar experiment evaluating defatted soybean meal was also undertaken (Table 4.30). In both experiments all the diets were fed at a pair-fed restricted level, determined by the fish's weight, though some significant food refusals occurred that complicated some of the outcomes of this study.

The inclusion of *L. angustifolius* kernel meal into the summit diet demonstrated that this form of lupin had considerable biological value when fed to *L. calcarifer* (Table 4.29). In comparison to the negative controls, a diet containing 40% of *L. angustifolius* kernel meal had a DGC of 1.65%/d, whereas the negative control diet with 40% of filler had a DGC of 1.02%/d, effectively showing a 62% advantage over filler at that inclusion level. The relative advantage of the *L. angustifolius* kernel meal increased with increasing inclusion levels, rising from a 30% advantage at 10% inclusion, to the 62% advantage at 40% inclusion.

In a separate trial, using larger fish, a diet containing 40% soybean meal had a DGC of 1.44%/d, whereas the negative control with 40% filler in that experiment had a DGC of only 0.49%/d. Effectively, this showed a massive 193% advantage of the soybean meal over the filler at that inclusion level. However, the growth attributable to the 40% filler treatment in this study was unusual when compared to that achieved from the same diet in other experiments and therefore casts doubts of the relative value observation of the soybean meal at the 40% inclusion level. (Table 4.30). Notably, the nutritional advantage of soybean meal was not as prominent at lower inclusion levels, with a negative biological value recorded at the 10% inclusion levels relative to the corresponding negative controls and the 20% and 30% inclusion levels showing only 14% and 22% advantages respectively (Table 4.30).

Many of the aberrations in both the *L. angustifolius* kernel meal experiment and also the soybean meal experiment were reportedly due to significant differences in the observed feed intake. Marked declines in the observed feed intake by fish fed the *L. angustifolius* kernel meal diets were not observed until 70% inclusion of the meal. Notably, the palatability of the soybean meal diets was reduced with the inclusion of the meal at 60% or more of the diet. It was also identified that 78% of the variance in growth rate associated within both experiments was attributable to feed intake variation.

Assessment of the nitrogen retention values observed in each of the two experiments also showed similarities in the biological value of the two ingredients. With both ingredients, declines in the retention of nitrogen were

observed with as little as 30% inclusion of either ingredient into the reference diet (Table 4.29 and Table 4.30). When these retention characteristics were compared against those observed from the negative control diets in each experiment, marked differences between experiments were seen. However, the attribution of the observed nitrogen retention effects becomes difficult given the disparity between responses by animals in each experiment to common diets. For example, in the *L. angustifolius* kernel meal experiment the fish fed the 40% filler diet had a nitrogen retention value of 36% compared with one of 24% observed for the same diet in the soybean meal experiment.

Williams (1998) also evaluated the retention of specific amino acids from the two ingredients. From this evaluation it was concluded that there was considerable preferential catabolism of specific amino acids in the diet. Notable was the retention of arginine, methionine and lysine. In most cases these three amino acids where observed to be retained at higher efficiencies than that of total dietary nitrogen. Retention of both methionine and lysine from diets with incremental inclusion of *L. angustifolius* kernel meal occurred at a rate greater than that observed from soybean meal. In contrast, arginine retention from *L. angustifolius* kernel meal was slightly lower than that observed from soybean meal. Evaluation of the levels of retention of these same amino acids from the filler diets supported that retention efficiency of these amino acids improved to about 108% that of the reference diet with minor reduction in total dietary protein content, but beyond 20% inclusion of filler that they too began to decline in efficiency to about 80% of that observed in the reference diet.

The retention of methionine from the *L. angustifolius* kernel meal diets was different from both soybean and the filler diets. Methionine retention efficiency improved to 147% of that of the reference diet at 50% inclusion of the *L. angustifolius* kernel meal, then declined to 134% efficiency at 70% inclusion. It was suggested that this response pattern of methionine indicated that this amino acid was the most limiting of the essential amino acids in these diets, and accordingly this ingredient.

Though the growth observations of the fish in these two experiments were considerably different, a comparative assessment of the *L. angustifolius* kernel meal and soybean meal experiments, based on the standardised assessment of the DGC of a common treatments, in this instance the reference diet, actually supported a high level of similarity in the biological value of the two protein meals. Each protein meal showing advantage or disadvantage relative to the other after standardisation, though generally the *L. angustifolius* kernel meal was marginally superior to the soybean meal.

It should be noted though, that considerable differences were observed between the two trials, even for common diets. In this regard it would be worthwhile to revisit this study to evaluate the comparative value of *L. angustifolius* kernel meal and soybean meal to this species.

Table 4.29 Performance characteristics of Asian seabass fed summit-diets evaluating L. angustifolius kernel meal. Data derived from Williams (1998).

	L. angustifolius kernel meal substitution									Diatomaceous earth substitution			
Parameter	Reference	10%	20%	30%	40%	50%	60%	70%	80%	10%	20%	30%	40%
Diet specifications (g/kg)													
Dry matter	933	933	932	932	932	932	931	931		939	944	950	956
Crude protein (g/kg DM)	523	516	508	501	493	486	478	471		471	418	366	314
Crude fat (g/kg DM)	158	149	141	132	123	115	106	97		142	126	111	95
Ash (g/kg DM)	74	70	66	62	58	55	51	47		166	257	349	440
Energy (MJ/kg DM)	22.82	22.60	22.37	22.15	21.93	21.71	21.48	21.26		20.54	18.26	15.97	13.69
Fish performance													
Initial weight (g)	121.9	121.1	121.4	115.9	117.3	120.0	124.4	119.2		121.7	122.9	121.4	116.8
Final weight (g)	187.0	191.1	191.2	191.9	174.5	176.8	171.8	146.6		173.9	183.0	170.0	150.4
DGC (%/d)	1.81	1.93	1.93	2.12	1.65	1.62	1.35	0.84		1.49	1.68	1.40	1.02
Feed intake (g/fish/d)	1.73	1.72	1.74	1.87	1.67	1.73	1.76	1.37		1.48	1.81	1.81	1.59
FCR (g fed / g gain)	1.12	1.03	1.04	1.04	1.23	1.30	1.57	2.10		1.20	1.27	1.57	1.99
Nitrogen retention (%)	31	38	35	35	33	29	24	24		35	33	39	36

Table 4.30 Performance characteristics of Asian seabass fed summit-diets evaluating soybean meal (defatted). Data derived from Williams (1998).

	Soybean meal (defatted) substitution									Diatomaceous earth substitution			
Parameter	Reference	10%	20%	30%	40%	50%	60%	70%	80%	10%	20%	30%	40%
Diet specifications (g/kg)													
Dry matter	927	925	923	921	919	918	916	914		933	940	946	952
Crude protein (g/kg DM)	521	521	520	520	519	519	518	518		469	417	365	313
Crude fat (g/kg DM)	151	137	124	110	96	83	69	55		136	121	106	91
Ash (g/kg DM)	78	77	77	76	76	75	74	74		169	260	352	443
Energy (MJ/kg DM)	22.18	22	22	21	21	21	21	20		20	18	16	13
Fish performance													
Initial weight (g)	151.5	152.3	159.2	149.1	153.9	153.1	150.2	148.5		155.0	152.3	147.9	154.6
Final weight (g)	219.0	223.5	231.8	209.6	212.1	206.4	191.0	182.3		229.6	213.0	196.6	173.2
DGC (%/d)	1.66	1.73	1.72	1.52	1.44	1.33	1.06	0.89		1.79	1.50	1.25	0.49
Feed intake (g/fish/d)	1.53	1.67	1.83	1.65	1.61	1.66	1.46	1.45		1.88	1.76	1.83	1.59
FCR (g : g)	0.95	0.99	1.06	1.16	1.16	1.32	1.50	1.80		1.06	1.22	1.58	3.60
Nitrogen retention (%)	41	42	43	34	36	32	29	19		42	42	36	24

4.5 Carp (*Cyprinus* spp.)

A limited amount of work has been reported on the use of lupins in diets for carp (*Cyprinus sp*.). A study by Viola et al. (1988) examined the use of whole-seed *L. angustifolius* (CP: 327 g/kg DM, CF: 60 g/kg DM; cultivar not stated) in diets for the common carp (*Cyprinus carpio*). In a series of two experiments, whole-seed *L. angustifolius* meal was added to a reference diet, first at a 30% inclusion level, then in a second experiment at a 45% inclusion level.

In the first experiment the lupin was included at the expense of both soybean meal (CP: 517 g/kg DM, CF: 11 g/kg DM) and sorghum (CP: 106 g/kg DM, CF: 31 g/kg DM). In the second experiment lupin was included at the expense of both fishmeal and sorghum, no soybean meal was included in either of the diets in the second experiment. In each case the diets were formulated to be iso-nitrogenous and iso-energetic on a gross basis, though notably the diet proximate specifications differed in each experiment (Table 4.31).

In the first experiment, 300 g carp fed the diets for 44 days showed significantly better growth when fed the 30% lupin diet (171.3 g gain) as opposed to those fed the reference diet (133.5 g gain) (Table 4.31). Feed conversion was also significantly better with the lupin diet (3.2 : 1 vs 4.1 : 1). No significant difference was observed in the composition characteristics of fish fed either of the diets. Similarly, both nitrogen and energy retention were also unaffected by diet in this experiment. While reasons for the superiority of the lupin based diet in this experiment were not clear, it was suggested that differences in the utilisation of the carbohydrate fraction of the diets may have been a factor. It is more likely that the better performance may have been related to higher digestible protein content of the lupin based diet, and that the reference diet was limiting in protein to start with. Reports of superior protein digestibility of lupin meal compared to soybean meal have been reported in many species (Hughes, 1988; Burel et al., 1998; McMeniman, 1998; Allan et al., 1998a)

In the second experiment, 225 g carp were fed the two experimental diets, a reference diet and a diet with 45% lupin inclusion, for 41 days. In this experiment there were no significant differences in either growth or food utilisation parameters (Table 4.31). Fish fed the reference diet had slightly higher levels of body fat at the end of the study than those fish fed the 45% lupin diet, but other composition parameters remained unchanged. Corresponding to the higher fat levels in the control diet fed fish, was also a higher level of energy retention.

No other data was reported for the nutritive value of any lupin products when fed to Carp.

Table 4.31 *Incorporation of L. angustifolius whole-seed into practical diets for carp. Data derived from Viola et al. (1988).*

	Experiment 1		*Experiment 2*	
	Lupin	*Reference*	*Lupin*	*Reference*
Diet ingredients (g/kg)				
Herring meal (70% protein)	150	150	200	350
Lupin whole seed meal (29.8% protein)	300	-	450	-
Defatted soybean meal (45.0% protein)	-	180	-	-
Sorghum (9.3% protein)	395	515	80	380
Wheat	100	100	200	200
Dicalcium phosphate	25	25	50	50
Poultry oil	30	30	20	20
Diet proximate specifications				
Crude protein (g/kg DM)	282	280	321	333
Crude fat (g/kg DM)	88	70	66	60
Gross ash (g/kg DM)	67	68	102	125
Gross energy (MJ/kg DM)	19.87	19.45	19.02	18.56
Fish performance criteria				
Initial weight (g)	300	300	225	225
Final weight (g)	471.3	433.5	339.5	342
DGC (%/d)	2.47	1.99	2.18	2.22
FCR (g fed / g gain)	3.2	4.1	3.25	3.20
Nitrogen retention (%)	16.2	14.5	17.6	16.50
Energy retention (%)	12.4	11.6	14.0	16.30

4.6 Tilapia *(Oreochromis* spp.*)*

Some recent studies, largely as yet unpublished, have evaluated *L. angustifolius* whole-seed and kernel meals in diets for tilapia (*O. niloticus*). The data provided so far is essentially quite rudimentary, evaluating only crude biological value of the lupin in comparison to other more routinely used dietary ingredients. The majority of diets used for this species tend to be under specified for performance, often making interpretation of the results difficult

A study was conducted based on the use of a commercially used diet formulation for the red tilapia (*O. niloticus*, Stirling strain) (Petterson et al., 1998). Experimental diets were made in which 50% replacement of either the fishmeal or soybean meal components by *L. angustifolius* kernel meal was undertaken. An additional diet in which 25% replacement of both the soybean and fishmeal components was also included. The diets were formulated on an iso-nitrogenous and iso-energetic basis (Table 4.32).

Table 4.32 *Performance of red tilapia fed practical diets with L. angustifolius kernel meal substitution for fishmeal and soybean meals. Data derived from Petterson et al. (1998).*

	Reference	A	B	C
Diet ingredients				
Fishmeal	200	120	200	120
Soybean meal	200	240	100	120
L. angustifolius kernel meal	-	120	100	240
Wheat pollard	370	290	370	290
Remains (uniform across treatments)	230	230	230	230
Diet proximate specifications				
Crude protein (g/kg)	301	293	273	300
Crude fat (g/kg DM)	43	47	41	54
Fish performance criteria				
Initial weight (g)	55.3	55.0	55.0	55.3
Final weight (g)	231.7	226.9	237.4	238.3
DGC (%/d)	4.86	4.78	4.98	4.98
FCR (g fed / g gain)	1.53	1.54	1.46	1.48

Reference: commercial based diet. A: 50% replacement of fishmeal content of reference diet with *L. angustifolius* kernel meal. B: 50% replacement of soybean meal content of reference diet with *L. angustifolius* kernel meal.. C: 25% replacement of both the fishmeal and soybean meal content of reference diet with *L. angustifolius* kernel meal.

Table 4.33 *Performance of large tilapia fed diets with L. angustifolius replacing either soybean meal or fishmeal. Data derived from Petterson et al. (1998).*

	Reference	A	B
Diet ingredients			
Fishmeal	150	150	85
Soybean meal	150	75	85
L. angustifolius kernel meal	-	75	170
Rice bran	327	327	327
Corn meal	320	320	320
Remains (undefined)	53	53	13
Diet proximate specifications			
Crude protein (g/kg)	243	242	253
Crude fat (g/kg DM)	96	117	103
Fish performance criteria			
Initial weight (g)	239.3	239.7	238.7
Final weight (g)	527.6	531.7	527.4
DGC (%/d)	3.90	3.94	3.91
FCR (g fed / g gain)	2.30	2.30	2.30
Protein efficiency ratio	1.65	1.68	1.59
Sensory evaluation			
Colour	3.37	3.23	2.56
Flavour	3.66	3.40	2.30
Texture	3.33	3.30	2.33
Acceptability	3.46	3.23	2.20

Reference: commercial based diet. A: 50% replacement of soybean meal content of reference diet with *L. angustifolius* kernel meal. B: 43% replacement of both the fishmeal and soybean meal content of reference diet with *L. angustifolius* kernel meal.

The diets were fed to the tilapia for 60 days. After this period similar growth and conversion performance were observed from all treatments (Table 4.32). This trial demonstrated that in low-specification diets for tilapia, the partial replacement of fishmeal and/or soybean could be effectively achieved with *L. angustifolius* kernel meal.

A second study by these same workers examined the use of *L. angustifolius* kernel meal with larger red tilapia (*O. niloticus*, Stirling strain) (Petterson et al., 1998). In this second study *L. angustifolius* kernel meal was used to replace 50% of the soybean meal in another reputed commercial diet formulation, with a third treatment consisting of 43% replacement of the soybean meal and 43% replacement of the fishmeal components. As with the previous trial by these researchers, the diets were again formulated on an iso-nitrogenous and iso-energetic basis, though actual diet energy levels were not detailed (Table 4.33).

The results of this second trial confirmed those of the earlier one, by demonstrating the capacity of *L. angustifolius* kernel meal to effectively replace 50% of the soybean meal in diets for red tilapia. However, in the diet were 43% of the fishmeal was replaced by *L. angustifolius* kernel meal, a decline in the protein efficiency ratio was observed. In addition, sensory evaluation of the fish fed these diets is was deemed that the replacement of fishmeal by *L. angustifolius* kernel meal reduced the overall sensory value of the fish. However, whether this effect is actually attributable to the increased *L. angustifolius* kernel meal content or the reduction in fishmeal content cannot be ascertained from this study.

4.7 Milkfish

Petterson (2000) reported some otherwise unpublished data (J.H. Hutabarat, unpubl.) on the substitution of *L. angustifolius* kernel meal into diets for the milkfish (*Chanos chanos*). In these studies, *L. angustifolius* kernel meal was substituted for soybean meal at incremented levels from 0% to 100%, in a diet with a total protein content of 330 g/kg. The size of the increments was not defined.

Performance of the fish fed the diets apparently improved with increasing inclusion of the of *L. angustifolius* kernel meal. The best performance of milkfish fed the experimental diets was observed from those fed the 100% *L. angustifolius* kernel meal diet, where the fish had an FCR of 3.5:1and a specific growth rate 2.0 %/d. Digestible protein and energy content of this diet were reported as 63% and 72% respectively. A second trial, in which the diets were made to a 350 g/kg crude protein level, was also undertaken on a similar basis with apparently similar results to those observed in the first study, with the *L. angustifolius* kernel meal diets outperforming those based on either soybean meal or *L. angustifolius* whole-seed meal. No specific details of this study were provided.

4.8 Turbot

Work reported by Burel et al. (2000a) examined the nutritional value of extruded *L. albus* kernels, extruded peas and both solvent-extracted and heat-treated rapeseed (*Brassica rapus*) meals in rainbow trout. These same workers also reported in that study, additional work examining the same test ingredients when fed to the marine flatfish, turbot (*Psetta maxima*). As with the rainbow trout study, the apparent digestible dry matter, protein (as N x 6.25), energy and phosphorus of these ingredients were also studied relative to a fishmeal reference (Table 4.34).

The studies with turbot by Burel et al. (2000a) found significantly higher apparent protein digestibility of *L. albus* kernel meal, pea and heat treated-rapeseed meals in comparison to the solvent extracted rapeseed meal. In addition, apparent energy digestibility of the *L. albus* kernel meal was also significantly higher than that of pea meal and the solvent-extracted rapeseed meal treatments, though not the heat-treated rapeseed meal. The phosphorus digestibility was essentially 100% in both the *L. albus* kernel meal and the pea meal, being significantly higher than both the rapeseed meals, in which the phosphorus content was only about as half as efficiently digested. It was suggested, however, that the

Table 4.34 Proximal composition and nutritional value of various plant meals to fed turbot. Data derieved from Burel et al. (2000a)

	Extruded peas	Extruded lupin	SE-Rapeseed	HT-Rapeseed
Ingredient Proximate Composition				
Dry matter (g/kg)	909	928	937	915
Crude protein (g/kg DM)	260	434	431	433
Crude fat (g/kg DM)	4.5	100	48	9
Ash (g/kg DM)	33	46	79	82
NFE (g/kg DM)	612	348	379	391
Phosphorus (g/kg) DM)	4.4	5.4	14.9	15.6
Nutrient Apparent Digestibility				
Dry matter (%)	71.5	80.5	57.1	64.6
Protein (%)	92.9	97.8	82.9	91.9
Energy (%)	77.7	85.1	69.3	80.9
Phosphorus (%)	100	100	49.3	64.7

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

high phosphorus apparent digestibilities in the pea and *L. albus* kernel meals were probably over-estimates, a consequence of leaching from faeces collected in a settlement column. Regardless, the data supported that phosphorus from both pea and *L. albus* kernel meals, were considerably better absorbed than that from rapeseed meals. In contrast to the results obtained by these workers with rainbow trout (Burel et al., 2000a), there were also significant differences in the dry matter apparent digestibilities obtained from turbot, with *L. albus* kernel meal being significantly more digested than both rapeseed meal treatments, but not more so than the pea meal (Table 4.34).

In concluding their study these workers supported that of the four meal types (pea, *L. albus* kernel and rapeseed: solvent-extracted or heat-treated), that *L. albus* kernel meal had the most promise as a valuable feed ingredient for turbot. While pea meal was used well, its use would be limited by its lower protein content.

In a follow up study to the nutritional assessment work undertaken by Burel et al. (2000a) with turbot and rainbow trout, these workers also examined the biological value of *L. albus* kernel and rapeseed meal as fed to turbot (Burel et al., 2000b).

In this second study (Burel et al., 2000b) included extruded *L. albus* kernel meal into two separate iso-nitrogenous and iso-energetic diets at 300 g/kg and 500 g/kg (Table 4.35). The performance of juvenile turbot when fed these diets was compared against a fish meal based reference diet of similar nutrient composition, with further additional diets examining the value of rapeseed meal to this species.

Growth by the turbot was best on the diet containing 300 g/kg of *L. albus* kernel meal, which was significantly better than that achieved with either of the rapeseed meal diets, but only numerically higher than the reference diet. Notably, both of the *L. albus* kernel meal diets had better food conversion than the reference diet, though only the diet with a *L. albus* kernel meal at a level of 300 g/kg had significantly better conversion (Table 4.35).

The turbot fed the *L. albus* kernel meal diets also had significantly higher nitrogen and phosphorus retention than that observed from turbot fed either of the rapeseed meal diets or the fishmeal based reference diet. These observations were highly consistent with the earlier work by these researchers who observed similar effects with rainbow trout (Burel et al., 1998) and were also consistent with observations from their digestibility studies (Burel et al., 2000a). There are considerable implications of these observations of the higher levels of both digestibility and nutrient retention in terms of the total potential environmental impact from intensive aquaculture.

In addition to the more common parameters of nutritional and biological evaluation reported by these researchers, further work on the plasma levels of triiodothyronine (T_3) and thyroxin (T_4) and the activities of some of the deiodinases, were also reported (Burel et al., 2000b).

What the outcomes of this additional work demonstrated was that although rapeseed meal had defined influences on the metabolism of both T_3 and T_4 and the deiodination pathways, similar effects were not observed with the use of *L. albus* kernel meal in the diet of turbot. Indeed the T_3 and T_4 levels and the deiodination pathways were comparable to those observed from fish fed the fishmeal based reference diet, although slightly higher T_3 levels were observed from fish fed the diet containing 300 g/kg of *L. albus* kernel meal. The specific implications of these observations were not discussed other than their general relation between increased metabolic rates and growth rates of the fish.

Table 4.35 Performance of turbot (Psetta maxima) fed L. albus kernel meal or canola meal

	Reference	*L. albus* 300	*L. albus* 500	Rapeseed 300	Rapeseed 460
Ingredients					
Extruded *L. albus* kernel meal	-	300	500	-	-
Rapeseed meal	-	-	-	300	460
Fish meal	590	440	345	460	370
Extruded peas	230	93	-	50	-
Fish oil	100	87	75	110	90
Remains	80	80	80	80	80
Diet proximate specifications					
Dry matter (g/kg)	885	894	893	888	892
Crude protein (g/kg DM)	530	526	525	522	516
Crude fat (g/kg DM)	170	173	172	184	146
Nitrogen-free extractives (g/kg DM)	85	158	206	168	228
Gross phosphorus (g/kg DM)	18	15	14	19	18
Gross ash (g/kg DM)	121	104	93	119	109
Gross energy (MJ/kg DM)	22	22.3	22.4	22.3	21.9
Fish performance criteria					
Initial weight (g)	48.6	47.2	50.2	49.6	48.7
Final weight (g)	122.9	127.5	122.5	113.5	102.2
DGC (%/d)	2.13	2.29	2.06	1.88	1.65
Feed intake (g/d/fish)	1.17	1.11	1.05	1.02	0.93
FCR (g/g)	0.98	0.85	0.91	0.99	1.08
N retention (%)	30.4	35.7	32.8	30.9	28.3
P retention (%)	32.9	45.2	44.4	31.9	35.7

4.9 Shrimp *(Penaeid sp.)*

Several published studies have examined the use of lupins in diets for shrimp, but only for a single species, *Penaeus monodon*, the giant tiger prawn. However, a range of approaches for evaluating the nutritional and biological value of lupins when fed to shrimp have been adopted.

The nutritional value of lupins to shrimp/prawns has been shown to be similar to that of most fish species (Table 4.36; Smith et al., 2000). As has been observed from most other fish species, the apparent digestibility values of dry matter, protein and energy were all higher in *L. angustifolius* kernel meal relative to that of the whole-seed meal. The nutritional value of the *L. angustifolius* kernel meal was generally similar to that of soybean meal, with marginally higher apparent protein digestibilities (94% vs 92%), though marginally lower apparent energy digestibilities (68% vs 71%). Similar in nutritional value to both *L. angustifolius* kernel meal and soybean meal was field pea meal, which had slightly higher apparent energy digestibilities still (83%), consistent with the effective digestion of the higher starch content of this protein resource.

Table 4.36 Proximal composition and nutritional value of various plant meals to fed the prawn, Penaeus monodon (based on data from Smith et al., 2000)

	L. angustifolius[a]		Soybean[b]	Canola[c]	Field pea[d]	Fishmeal
	Whole-seed	Kernel meal				
Ingredient proximate composition						
Crude protein (g/kg DM)	341	448	478	366	255	732
Crude fat (g/kg DM)	57	71	37	26	11	99
Ash (g/kg DM)	28	35	80	80	34	142
NFE (g/kg DM)	574	446	405	528	700	27
Gross energy (MJ/kg DM)	17.9	20.6	17.0	19.9	17.0	21.3
Nutrient Apparent Digestibility						
Dry matter (%)	39	67	67	49	72	86
Protein (%)	88	94	92	79	89	93
Energy (%)	45	68	71	53	83	89

[a]*L. angustifolius* cv. Gungarru ; [b]Solvent extracted soybean meal; [c]Solvent extracted canola meal; [d]*Pisum sativum* cv. Dunn

Sarac et al. (1998) undertook a series of studies to determine the biological value of several forms of the lupin *L. angustifolius* (cv. Gungurru). Using a summit-dilution approach, whole-seed and kernel meals and a protein concentrate were incorporated into a summit diet at incremented levels from 10% to 70%, at 10% increments. A series of control diets, were diatomaceous earth was added into the summit diets at 10%, 20%, 30% and 40% were also included as treatments in the study (Table 4.37 to 4.39).

The inclusion of *L. angustifolius* kernel meal into the summit diet showed that this form of lupin has considerable biological value (Table 4.37). In comparison to the negative controls shrimp fed a diet containing 40% of *L. angustifolius* kernel meal had a growth rate (DGC) of 0.90%/d, whereas shrimp fed the negative control with 40% filler had a DGC of 0.52%/d. The difference between these two treatments effectively showing a 73% advantage of the *L. angustifolius* kernel meal over the filler at that same inclusion level. Using this comparison to the relative negative control, the relative advantage of the *L. angustifolius* kernel meal was observed to increase with increasing inclusion levels, rising from a 9% advantage at 10% inclusion, to a 73% advantage at 40% inclusion. In comparison, shrimp fed a diet containing 40% soybean meal had a DGC of 1.02%/d, whereas those fed the negative control with 40% filler in that experiment had a DGC of 0.81%/d, thus effectively only showing a 26% advantage over those shrimp fed the diet with filler at that same inclusion level (Table 4.40). The nutritional advantage of soybean meal was not evident at lower inclusion levels, with a negative comparative biological value of the diets recorded at 10%, 20% and 30% soybean meal inclusion levels relative to the corresponding negative (filler) control diets.

Though the growth rates (DGC) of shrimp fed each of the 40% filler diets were considerably different, comparative assessment was made based on the standardised assessment of common treatments, such as the 40% filler diet. Notable from the soybean inclusion treatments were the relatively high feed conversion ratios, substantially more so than those observed even for the diets with corresponding levels of dietary filler. It was suggested that this unexpectedly poor performance of the soybean meal may have been attributable to contamination of the soybean meal with aflotoxin or pesticides. Other contributing factors such as high levels of phytate and lipoxidases were also suggested (Sarac et al., 1998).

Figure 18. Several studies have identified good potential for lupins in shrimp diets.

Inclusion of *L. angustifolius* whole-seed meal into the summit diet showed that this form of lupin had less biological value than that of the lupin kernel meal, but more than that of the soybean meal (Table 4.38). In comparison to the negative controls, shrimp fed a diet containing 40% of *L. angustifolius* whole-seed meal had a DGC of 0.80%/d, whereas those shrimp fed the negative control with 40% filler had a DGC of 0.52%/d, effectively showing a 54% advantage over the filler at that inclusion level.

Conversely, inclusion of *L. angustifolius* protein concentrate into the summit diet showed that this form of lupin had considerably less biological value than that of the lupin kernel meal, and even less that that of the both the whole-seed and soybean meals (Table 4.39). In comparison to the negative controls, shrimp fed a diet containing 40% of *L. angustifolius* protein concentrate had a DGC of 0.72%/d, whereas shrimp fed the negative control with 40% filler had a DGC of 0.62%/d, effectively showing only a 16% advantage over filler at that inclusion level. It was suggested that problems with nutrient deficiencies might have been present with the inclusion of the lupin protein concentrate, specifically a limitation in dietary methionine requirements. It was suggested that the lupin protein concentrates could be effectively used in diets for prawns up to an inclusion level of 20%. It would be interesting to reevaluate this work with the supplementation of the diets with crystalline amino acids to counter suspected deficiencies.

Sudaryono et al. (1999a) examined the biological value of *L. albus* whole-seed and kernel meals (cultivar not defined), solvent extracted soybean meal, *L. angustifolius* kernel meal (cultivar not defined) and a lupin protein concentrate (species nor cultivar not defined). Each of the test ingredients was incorporated into experimental diets at inclusion levels varying between 240 g/kg and 400 g/kg, depending on the ingredient, in an attempt to maintain total dietary crude protein levels at 400 g/kg (Table 4.41).

In this study, significantly better growth was observed of shrimp fed the *L. angustifolius* kernel and soybean meals, than that from shrimp fed any of the diets containing *L. albus* whole-seed and kernel meals or lupin protein concentrate (Table 4.41). It was suggested that this was a likely response to superior protein utilisation from the available (digestible) protein in each of the *L. angustifolius* kernel meal and soybean meal diets. Concomitant with this superior utilisation of *L. angustifolius* kernel and soybean meal protein was also a significantly higher feed intake of these same two diets (Table 4.41). It was stated that the value of the *L. angustifolius* kernel meal was equivalent to that of soybean meal (Sudaryono et al., 1999a), though it should be noted that this would, rationally, only be on an equivalent protein to protein basis.

Table 4.37 Performance characteristics of the prawn, Penaeus monodon fed summit-diets evaluating L. angustifolius kernel meal. Data derived from Sarac et al. (1998).

	L. angustifolius kernel meal substitution								Diatomaceous earth substitution			
	Reference	10%	20%	30%	40%	50%	60%	70%	10%	20%	30%	40%
Diet specifications												
Dry matter (g/kg)	917	917	924	921	915	916	920	919	925	931	934	934
Crude protein (g/kg DM)	487	481	473	467	468	461	451	444	426	377	323	279
Crude fat (g/kg DM)	114	115	117	120	123	123	125	131	103	95	96	90
Gross ash (g/kg DM)	112	114	102	96	86	80	73	66	98	96	84	77
Nitrogen-free extract (g/kg DM)	286	290	308	317	323	336	351	360	373	432	498	554
Energy (MJ/kg DM)	22.60	22.54	22.32	22.49	22.95	22.98	22.91	23.09	21.76	n/s	21.23	20.92
Prawn performance criteria												
Initial weight (g)	2.48	2.48	2.48	2.48	2.48	2.48	2.48	2.48	2.48	2.48	2.48	2.48
Final weight (g)	6.55	6.20	5.89	5.44	5.21	5.03	4.81	4.79	5.79	5.34	4.52	3.87
DGC (%/d)	1.23	1.15	1.08	0.96	0.90	0.86	0.80	0.79	1.05	0.94	0.71	0.52
FCR (g fed / g gain)	1.71	1.83	1.78	2.02	2.24	2.67	3.48	7.91	2.10	1.83	2.61	3.49
Feed intake (g/prawn/d)	0.128	0.134	0.158	0.153	0.149	0.144	0.132	0.112	0.142	0.176	0.161	0.160
Survival (%)	83	83	92	83	92	92	100	83	67	92	83	100

n/s: not stated.

Table 4.38 Performance characteristics of Penaeus monodon fed summit-diets evaluating L. angustifolius whole-seed meal. Data derived from Sarac et al. (1998).

	L. angustifolius whole-seed meal substitution								Diatomaceous earth substitution			
	Reference	10%	20%	30%	40%	50%	60%	70%	10%	20%	30%	40%
Diet specifications												
Dry matter (g/kg)	917	936	931	931	928	927	933	934	925	931	934	934
Crude protein (g/kg DM)	487	424	432	419	411	393	374	355	426	377	323	279
Crude fat (g/kg DM)	114	109	110	112	114	114	115	116	103	95	96	90
Gross ash (g/kg DM)	112	109	101	94	89	82	71	64	98	96	84	77
Nitrogen-free extract (g/kg DM)	286	357	357	374	386	411	441	464	373	432	498	554
Energy (MJ/kg DM)	22.60	21.47	21.72	21.75	22.05	22.05	21.53	21.97	21.76	n/s	21.23	20.92
Prawn performance criteria												
Initial weight (g)	2.48	2.48	2.48	2.48	2.48	2.48	2.48	2.48	2.48	2.48	2.48	2.48
Final weight (g)	6.55	6.21	6.26	5.61	4.83	4.85	4.28	4.58	5.79	5.34	4.52	3.87
DGC (%/d)	1.23	1.15	1.17	1.01	0.80	0.81	0.64	0.73	1.05	0.94	0.71	0.52
FCR (g fed / g gain)	1.71	1.6	1.55	1.85	2.2	2.79	4.12	5.07	2.10	1.83	2.61	3.49
Feed intake (g/prawn/d)	0.128	0.161	0.161	0.160	0.169	0.144	0.139	0.119	0.142	0.176	0.161	0.160
Survival (%)	83	92	91	67	100	92	67	83	67	92	83	100

n/s: not stated.

Table 4.39 *Performance characteristics of Penaeus monodon fed summit-diets evaluating L. angustifolius protein concentrate. Data derived from Sarac et al. (1998).*

	L. angustifolius protein concentrate substitution						Diatomaceous earth substitution			
	10%	20%	30%	40%	50%	60%	Reference (0%)	10%	20%	30%
Diet specifications										
Dry matter (g/kg)	915	909	908	922	915	919	914	910	933	930
Crude protein (g/kg DM)	502	503	507	496	499	498	486	437	367	314
Crude fat (g/kg DM)	120	128	133	133	133	133	123	110	107	95
Gross ash (g/kg DM)	110	102	95	88	81	72	119	211	271	374
Nitrogen-free extract (g/kg DM)	268	266	266	283	287	296	273	242	255	217
Energy (MJ/kg DM)	22.80	23.11	23.19	22.84	23.19	23.32	22.62	20.16	16.79	14.62
Prawn performance criteria										
Initial weight (g)	4.30	4.30	4.30	4.30	4.30	4.30	4.30	4.30	4.30	4.30
Final weight (g)	8.39	8.57	7.67	7.19	6.64	5.01	8.24	7.80	7.59	6.72
DGC (%/d)	0.97	1.00	0.82	0.72	0.60	0.20	0.94	0.85	0.81	0.62
FCR (g fed / g gain)	2.11	1.94	2.43	2.87	2.87	4.05	1.97	1.93	2.18	2.35
Feed intake (g/prawn/d)	0.144	0.143	0.156	0.159	0.182	0.213	0.160	0.190	0.180	0.208

Table 4.40 Performance characteristics of the prawn, Penaeus monodon fed summit-diets evaluating soybean meal. Data derived from Sarac et al. (1998).

	Soybean meal substitution								Diatomaceous earth substitution			
	Reference	10%	20%	30%	40%	50%	60%	70%	10%	20%	30%	40%
Diet specifications												
Dry matter (g/kg)	923	940	902	916	929	921	927	919	941	956	960	962
Crude protein (g/kg DM)	502	485	509	522	489	489	485	489	431	365	305	252
Gross ash (g/kg DM)	119	116	120	115	110	110	106	104	205	298	345	425
Prawn performance criteria												
Initial weight (g)	2.49	2.49	2.49	2.49	2.49	2.49	2.49	2.49	2.49	2.49	2.49	2.49
Final weight (g)	7.28	6.38	5.75	5.54	5.66	5.55	5.35	5.34	7.92	6.67	6.35	4.89
DGC (%/d)	1.39	1.19	1.04	0.99	1.02	0.99	0.94	0.93	1.52	1.25	1.18	0.81
FCR (g fed / g gain)	1.85	2.59	2.32	2.55	1.67	2.23	2.43	2.94	1.36	1.77	1.81	1.53
Feed intake (g/prawn/d)	0.211	0.240	0.180	0.185	0.126	0.163	0.165	0.200	0.176	0.176	0.166	0.088
Survival (%)	83	42	58	42	66	58	66	75	75	66	92	66

The poor utilisation of the lupin protein concentrate was attributed to the influence of the protein extraction process on the quality of the protein content (Table 4.41). It has been reported that changes to the chemical structure of some of the protein fractions (eg. globulins and albumins) due to the extraction process, does occur (Petterson, 2000), and that these may have influenced the value of the protein content in the lupin protein concentrate used in this study. It may have been of value, in this essence to have also evaluated some soybean protein concentrates in the study, for comparative purposes. Similarly a comparative nutritional evaluation of the lupin protein concentrate against other plant protein resources is warranted.

Sudaryono et al. (1999a) also suggested that the poorer performance of shrimp fed the *L. albus* kernel meal relative to that of *L. angustifolius* kernel meal was reflective of the differences in feed intake of the two diets (Table 4.41). No explanations were offered as to why differences in intake were observed between these two treatments, though similar observations have been reported from studies comparing the inclusion of *L. albus* meals to that of *L. angustifolius* meals in diets fed to pigs (Kemm et al., 1987). These studies also did not identify the causative agent for reduced feed intake by the pigs.

The *L. albus* kernel meal did not support significantly better performance of prawns, than that of prawns fed the *L. albus* whole-seed diet (Table 4.41). Though in all parameters the performance was numerically higher. It would be of value to reconsider this work with an increase in experimental power in the design (Searcy-Bernal, 1994). Other studies have identified that four or more replicates, each with at least five animals are required in nutritional studies with this species to have reasonable confidence in detecting significant effects (Glencross et al., 1999).

A reduction in the digestibility of the dry matter of the diet was observed with the inclusion of *L. albus* whole-seed meal, relative to the other experimental diets (Table 4.41). It was suggested that this could have been a response to increased levels of fibre present in the diet (Table 4.41). Other workers have also reported deterioration in the dry matter apparent digestible value of diets, concomitant with increased levels of fibre in the diet (Akiyama et al., 1989; Catacutan, 1991). No significant differences were observed of the apparent digestibility of protein between any of the diets. This observation was inconsistent with the observations of the dry matter digestibilities (Table 4.41).

That no growth effects were evident from the differences in digestible values observed in this study confirms that most of the diets were probably over specified for protein, thereby reducing the possibility of observing growth effects attributable to inclusion of specific ingredients.

In a second study by these same authors (Sudaryono et al., 1999b), the incremental inclusion of *L. albus* kernel meal into a reference diet in place of soybean meal was reported (Table 4.42). The test diets represented a 25%, 50%, 75% and 100% replacement of the dietary soybean meal. In this study, the diets were formulated to be iso-

Table 4.41 *Utilisation of soybean, L. albus whole-seed and kernel meals and L. angustfolius kernel meal and lupin protein concentrate by the tiger prawn, Penaeus monodon. Data derived from Sudaryono et al. (1999a).*

	Soybean	*L. albus*		*L. angustifolius*	
		whole-	kernel	kernel	concentrate
Diet ingredients (g/kg)					
Soybean meal	300	-	-	-	-
L. albus whole-seed meal	-	400	-	-	-
L. albus kernel meal	-	-	350	-	-
L. angustofolius kernel meal	-	-	-	360	-
L. angustofolius protein concentrate	-	-	-	-	240
Wheat flour	215	185	235	225	285
Rice bran	50	-	-	-	50
Fish oil	20	-	-	-	10
Remains (uniform across treatments)	415	415	415	415	415
Diet proximate specifications					
Crude protein (g/kg DM)	415	410	429	417	425
Crude fat (g/kg DM)	79	86	96	82	107
Gross ash (g/kg DM)	79	86	96	82	107
Crude Fibre	43	66	45	45	33
NFE	366	355	390	383	340
Gross Energy	19.28	19.23	20.64	19.71	20.12
Lysine (g/ 160 g N)	58.7	46.8	43.2	53.6	33.8
Methionine (g/ 160 g N)	21.0	17.1	17.1	19.2	17.8
Prawn performance criteria					
Initial weight (g)	4.08	4.13	4.03	4.03	4.07
Final weight (g)	7.62	6.51	6.46	7.52	6.99
DGC (%/d)	0.88	0.63	0.64	0.87	0.75
FCR (g fed / g gain)	2.3	2.9	2.8	2.3	2.7
Feed intake (g/prawn/d)	0.196	0.165	0.164	0.190	0.186
N retention	23.7	20.0	20.5	24.5	20.7
Apparent Protein Digestibility (%)	87.5	87.1	87.7	89.1	87.9

Table 4.42 *Replacement of soybean meal with L. albus kernel meal in diets fed to the prawn, Penaeus monodon. Data derived from Sudaryono et al. (1999b).*

	Soybean meal replacements level (%)				
	0	25	50	75	100
Diet ingredients (g/kg)					
Soybean meal	300	225	150	75	0
L. albus kernel meal	0	90	170	260	350
Wheat flour	185	200	225	230	225
Rice bran	90	60	30	10	0
Remains (uniform across treatments)	425	425	425	425	425
Diet proximate specifications					
Crude protein (g/kg DM)	404	401	394	397	387
Crude fat (g/kg DM)	75	77	79	87	95
Gross ash (g/kg DM)	144	135	129	126	120
Crude Fibre (g/kg DM)	44	42	41	40	41
NFE (g/kg DM)	333	345	357	350	357
Gross Energy (MJ/kg DM)	18.26	18.47	18.60	18.86	19.06
Lysine (g/ 160 g N)	67	65	63	62	61
Methionine (g/ 160 g N)	20	20	19	19	18
Prawn performance criteria					
Initial weight (g)	4.40	4.32	4.38	4.41	4.27
Final weight (g)	8.22	8.02	7.84	7.49	6.73
DGC (%/d)	0.90	0.89	0.83	0.75	0.63
FCR (g fed / g gain)	2.30	2.26	2.54	2.72	2.97
Feed intake (g/prawn/d)	0.207	0.199	0.209	0.199	0.173
Survival (%)	87	87	87	87	87
N retention (%)	20.3	20.9	20.8	16.6	15.6

nitrogenous and iso-energetic, though notably only on a gross basis. Interestingly, the dietary lysine and methionine levels remained relatively constant, despite large levels of substitution of soybean meal for *L. albus* kernel meal, without the use of supplemental crystaline amino acids. These inconsistencies were not explained.

Growth performance of prawns fed the experimental diets supported that *L. albus* kernel meal could only effectively replace up to 50% of the soybean meal in the diet (Table 4.42). These observations were consistent with what was reported by these workers in an earlier study (Sudaryono et al., 1999a), where they observed the nutritional and biological value of *L. albus* kernel meal to be less than that of soybean meal. In light of the findings of this earlier study it was interesting that these workers chose to use *L. albus* kernel meal in the second study, when *L. angustifolius* kernel meal had been shown to be superior to not only *L. albus* kernel meal, but also soybean meal. Consistent with what was reported in the earlier study, the food conversion ratios and nitrogen retention values also deteriorated with increased inclusion of *L. albus* kernel meal in the diet. Whilst there was some correlation of the deterioration in these performance criteria with a declining feed intake, this too was not conclusive.

Figure 19. Meals, particularly those of the kernels, of both L. angustifolius and soybean have been shown to provide good nutritional value in shrimp diets

4.10 Freshwater crayfish

Considerable development of freshwater crayfish culture has taken place in Australia in recent years (Morrissy et al., 1995; Lawrence 1998). The largest industry sector is for production of the yabby, *Cherax albidus*, although other species such as *C. destructor, C. tenuimanus* and *C. quadricarinatus* are also produced. Typically, the production systems and feeds used with these species are low intensity and trophic demands are also not high. Feeds are considerably less specified than those used in the prawn farming sector and accordingly cost efficiency of feed use has been more paramount than production efficiency. Comparatively limited work has been undertaken examining the development of specific feeds for any of the *Cherax* spp., let alone the use of lupins in their diets. The greatest use of lupins in this sector has been within that of the yabby industries, notably in the diets of *C. albidus*.

4.10.1 Yabbies

Considerable work has been undertaken by Lawrence et al. (1998; 2000) in which the use of *L. albus* as a feed for *C. albidus* has been reported. In these studies *L. albus* whole seed, rolled and cracked, was used as a whole feed, fed at a range of provision rates and was also compared against a range of other commodities and compound feeds. Of particular note in these studies is that they were conducted in replicated (n=3) pond studies, thereby maintaining particular relevance to industry practices.

Initial studies (Lawrence et al., 1998), examined the use of *L. albus* whole seed, rolled and cracked and fed at 2.5 g / m^2/ week and was compared to an unfed control (Table 4.43). Growth by yabbies in this treatment was significantly faster than that of those from the unfed treatment, though there were no significant differences in survival between the two treatments. These findings support that the use of *L. albus* whole seed, rolled and cracked, supports production of yabbies to a higher production rate than that obtained from natural productivity within the ponds. The comparatively high level of growth seen in the unfed control gives strong indication of the level of natural productivity available within the pond systems used in these studies.

Table 4.43 Growth and survival of yabbies (Cherax albidus) fed either L. albus rolled whole-seed (2.5 g/ m^2/ week) or unfed. Data derived from Lawrence et al. (1998).

	Unfed	*L. albus* whole-seed rolled
Initial weight (g)	19.4	19.4
Final weight (g)	32.8	38.9
DGC (%/d)	0.49	0.67
Survival (%)	69	68

A second study (Lawrence et al., 1998), also in pond systems, examined the comparative value of *L. albus* whole seed, rolled and cracked, and fed at two rates (2.5 g / m^2/ week and 5.0 g / m^2/ week) and was compared to the use of a Crayfish Reference Diet (CRD) designed for freshwater crayfish research (Morrissy, 1992; Lawrence et al., 1998). The CRD was fed at 2.5 g / m^2/ week.

Growth by yabbies fed the CRD was significantly faster than that of yabbies fed *L. albus* whole seed, rolled and cracked, and fed at the same feed rate (2.5 g / m^2/ week). However, the growth was not significantly faster than that of the yabbies fed the lupins at 5.0 g / m^2/ week (Table 4.44). This suggests that the CRD was more efficient on a weight for weight basis. However, that the CRD contains 18% lupins supports that considerable potential still exists for lupins as as an ingredient of formulated crayfish diets (Lawrence et al., 1998). It would have been of value if this study had further quantified both total nitrogen and energy flows within the pond ecosystem to ascertain if the predominant factor driving production was nitrogen or energy. Similarly, studies to assess whether the yabbies were actually eating the lupins,or whether the lupins were actually supporting improved productivity through increasing the natural productivity would also be of value.

Notably, survival of both of the two lupin fed treatments was not significantly different than that obtained from yabbies fed the compound, crustacean reference diet (Table 4.44).

Economic analyses (C. Lawrence, unpublished data) confirm that feeding yabbies the crayfish reference diet is more profitable than providing lupins. It should be noted that successful formulated feeds for yabbies in dams may contain about 20% lupin meal (Lawrence and Morrissy, 2000).

Table 4.44 *Growth and survival of yabbies (Cherax albidus) fed either of two feed rates of L. albus rolled whole-seed or a practical crustacean reference diet.*

	Treatment 1	Treatment 2	Treatment 3
Diet feed rates (g/m^2/week)			
Crayfish reference diet	2.5	-	-
L. albus whole-seed rolled	-	2.5	5
Performance criteria			
Initial weight (g)	19.5	19.5	19.5
Final weight (g)	53.4	47.7	51.6
DGC (%/d)	0.61	0.53	0.59
Survival (%)	56	79	75
FCR (g fed / g gain)	2.63	3.31	4.99

While these three studies provide a very basic account for the value of *L. albus* to yabbies when fed in an extensive pond system, they provide few meaningful data on the intrinsic nutritional value of lupins to these animals per se. The differences in technical approach between this series of studies and the style undertaken with prawns clearly show the level of nutritional understanding of either species. However, the practical relevance of either approach could easily be argued in favour of the style of work done with the yabbies, given the highly complex trophic systems involved with pond aquaculture, and the potential irrelevance of tank based data.

4.10.2 Marron

The amount of nutritional work that has been undertaken on marron (*Cherax tenuimanus*) is considerably greater than that that has been undertaken on yabbies (Morrissy, 1982; 1992). However, this does not extend to the amount of information available on the use of feed ingredients when fed to this species. To date the only available literature where lupins had been fed to marron is an abstract reviewed in Morrissy (1992).

A study (abstract by; Bennison and Morrissy, 1990) where lupin (assumed to be *L. angustifolius* whole-seed) meal was included in a standard reference diet at either or two inclusion levels was undertaken. Lupins were included in a pelleted reference diet at either 18% or 73% at the expense of a wheat component. The increase in lupin content of the marron feed was also noted to have increased the diet protein content from 225 g/kg AF, to 329 g/kg AF. The diets were used on a commercial marron farm being fed to pond reared marron. Details of the feeding regime were not supplied. Specific performance details of the marron fed the diets were not supplied, but it was stated that mean size, total biomass and survival for the two feed groups were essentially identical.

Few other studies involving marron being fed lupins have been reported.

Figure 20. Marron

4.11 Abalone *(Haliotis* spp.*)*

Abalone culture is a relatively new aquaculture industry that has relied heavily on seaweeds however, the use of compound diets has become more commercially acceptable, particularly in Australia (Fleming et al., 1996). Several studies have been published examining the nutritional value of several commonly used ingredients, including lupins, when fed to the greenlip abalone (*Haliotis laevigata*). No studies were identified where the biological value of lupins was reported.

Fleming et al. (1998), studying the greenlip abalone (*Haliotis laevigata*), reported the digestibility values of a diet in which *L. angustifolius* kernel meal (cv. Gungurru) represented 100% of the dietary protein and also several compounded diets in which this meal represented significant proportions of the total diet (Table 4.46). Key aspects of this study included the determination of nitrogen and energy digestibilities by this species, but also the digestibilities of key amino acids.

Assessment of the specific digestible value of the lupin kernel meal was made based on known characteristics of other potentially confounding ingredients, such as pregelled starch or casein. Nitrogen digestibility of the diet in which the lupin kernel meal represented all of the dietary nitrogen was 91%. Energy digestibility of this diet was 80%. Amino acid digestibilities were consistently high ranging from 86% for isoleucine to 95% for arginine. Lysine digestibility was 91%, methionine digestibility, surprisingly given the importance of this amino acid in diets formulated with lupin kernel meal, was not reported (Table 4.46). In comparison, a diet, in which all protein was provided by fishmeal, which notably had a much higher protein level, had substantially lower nitrogen (43%) and energy (51%) digestibilities. All amino acid digestibilities of the fishmeal diet were also substantially lower than that of lupin kernel meal diet (Table 4.46). No reasons were given for the low nutritional value of fish meal for this species. Though notably the total protein content of this diet was substantially higher than that reported to be optimal for this species (Coote et al., 2001).

Digestibilities of the assessed, compounded diets varied depending on the composition of the diets evaluated. Though each of the diets contained lupin kernel meal at the same inclusion level (300 g/kg), the addition of semolina, barley or both combined, along with casein had variable effects on the digestibility values observed. Dietary nitrogen digestibility substantially diminished (84%) with 400 g/kg inclusion of semolina and 158 g/kg inclusion of casein. Energy digestibility of this diet was also substantially reduced (64%) compared to the diet in which the lupin kernel meal represented the only protein source. In comparison, the diet with 400 g/kg inclusion of barley and 149 g/kg inclusion of casein had a nitrogen digestibility of 86%, marginally higher than that of the semolina compounded diet. In contrast though the energy digestibility was marginally lower at 57%. The diet in which 200 g/kg of each of semolina and barley and 153 g/kg of casein were added to 300 g/kg of lupin kernel meal had digestibility values intermediate of those in which just one of either semolina or barley was included with the lupin kernel meal. These findings support that lupin kernel meals have substantial nutritional value to abalone, considerably superior to that of other meals such as fishmeal.

Table 4.46 *Utilisation of L. angustifolius kernel meal and fishmeal by the greenlip abalone (Haliotis laevigata). Data derived from Fleming et al. (1998).*

	Lupin	*Fishmeal*	*Diet A*	*Diet B*	*Diet C*
Diet ingredients (g/kg)					
L. angustifolius kernel meal	700	-	300	300	300
Semolina	-	-	400	-	200
Barley	-	-	-	400	200
Fishmeal	-	700	-	-	-
Casein	-	-	158	149	153.5
Pregelled starch	264.2	264.2	106.2	115.2	110.7
Remains (uniform across treatments)	35.8	35.8	35.8	35.8	35.8
Diet proximate specifications					
Dry matter (g/kg)	907	936	932	913	910
Crude protein (g/kg DM)	261	496	302	297	302
Crude fat (g/kg DM)	73	91	31	28	30
Gross ash (g/kg DM)	32	123	30	21	27
Gross energy (MJ/kg DM)	19.32	19.88	19.41	19.34	19.36
Abalone digestibility criteria					
Nitrogen (%)	91	43	84	86	85
Energy (%)	80	51	61	57	60
Lysine (%)	91	42	87	88	86
Threonine (%)	89	36	83	85	83
Methionine (%)	n/d	n/d	n/d	n/d	n/d
Isoleucine (%)	86	33	83	84	80
Leucine (%)	88	36	84	86	83
Tryptophan (%)	n/d	n/d	n/d	n/d	n/d
Valine (%)	88	33	85	86	84
Phenylalanine (%)	89	34	84	86	83
Histidine (%)	91	48	88	89	87
Arginine (%)	95	37	87	90	85

n/d : not determined.

Fleming et al. (1998) in discussing the implications of their findings suggested that, unlike many other monogastric animals, abalone did not have problems with dealing with dietary NSP. It was stated that this was consistent with this species natural diet and that it reputedly had substantial levels of endogenous carbohydrases (Fleming et al., 1996). Little comment was made on the relative values of each of the protein resources evaluated by these workers.

Vandepeer et al. (1999), evaluated the nutritional value of a range of legume meals to the greenlip abalone and also examined the effects of autoclaving of the meals to mimic the influence of extrusion processing. Each of the legume meals was also included in another series of treatments, where each was supplemented with the exogenous enzyme phytase. The data on the phytase supplementation to the meals is not presented in Table 4.47. Legume meals included in the study were, *L. luteus* whole-seed meal, defatted soybean meal, Field pea meal, Vetch meal and Faba bean meal (Table 4.47). As with the study by Fleming et al. (1998), key aspects of this study also included the determination of nitrogen and energy digestibilities by this species. The digestibilities of key amino acids were also determined, though only those of lysine and methionine are presented here.

Assessment of the nitrogen digestibility of each of the ingredients clearly identified the *L. luteus* whole-seed meal as having the most digestible dietary nitrogen (91%). The level of nitrogen digestibility of the *L. luteus* whole-seed meal was significantly better than that of the soybean meal (87%), faba bean meal (85%) and the vetch and pea meals (both 75%). Energy digetibilities of the soybean meal and the *L. luteus* whole-seed meal were not significantly different from each other (84% and 83% respectively), though were considerably better that those of all the other meal types (Table 4.47). Dry matter digestibilties of the diets were best for the *L. luteus* whole-seed meal diet (61%), which was marginally better than that of the soybean meal diet (57%). Dry matter digestibilities of all the other diets were poor, typically being lower than 50%, with some as low as 25%. Digestibilities of lysine and methionine were consistently high ranging from 76% for to 92% for lysine. Highest lysine digestibility was observed from the *L. luteus* whole-seed meal, though this was not significantly better than that of the soybean meal. Lysine digestibilties of the other three meals were lower than that of both the soybean and the *L. luteus* whole-seed meals (Table 4.47). Methionine digestibility, was slightly better in the soybean meal than that of the *L. luteus* whole-seed meal (92% cf. 87%). Digestibilities of methionine in the other meals were generally lower, with faba bean meal being the exception (89%) (Table 4.47). Phosphorus digestibility was clearly best in the pea and faba bean meals (94% and 93% respectively), with each of the other meals, including the *L. luteus* whole-seed meal, having lower phosphorus digestibilities (84% to 86%) (Table 4.47).

Autoclaving of the meals had appreciable effects on the nutritive value of all of the meals examined in this study. Nitrogen digestibilties, along with those of the amino acids, were the

most significantly affected, with consistent decreases in digestibility values across all meals (Table 4.47). It was suggested that this may have been the result of Maillard reactions rendering much of the protein unavailable though the formation of amino-sugar complexes (van Barneveld 1993) occuring with the autoclaving of the meals. Contrasting this was the influence on energy digestibilities. Energy digestibilities of both the soybean and the *L. luteus* whole-seed meals decrease with autoclaving, though the digestibilties of the other three meals substantially improved (Table 4.47). It is suspected that this effect is attributable to the low starch levels in both the soybean and the *L. luteus* whole-seed meals, but appreciable levels in the vetch, faba bean and pea meals. Phosphorus digestibilities were unaffected by autoclaving (Table 4.47).

Though not presented in Table 4.47, it was noted that for the phytase supplemented treatments, only the level of phosphorus digestibility was improved in the *L. luteus* whole-seed meal. Improvements in the dry matter digestibilities of faba bean meal, vetch meal were also observed, as were some improvements in the digestibility of the nitrogen content of vetch meal, and some amino acids in pea meal, faba bean meal and vetch meal.

Vandepeer et al. (1999) in discussing the implications of their findings suggested that *L. luteus* whole-seed meal was a suitable feedstuff for use in artificial diets for abalone. These workers also suggested that future studies on legume use in the diets of abalone needed to address issues associated with inclusion levels and processing time and temperatures.

An accessory study to that of Vandepeer et al. (1999) was reported by Kemp et al. (1999). The same treatments as used by Vandepeer et al. (1999) were sampled to assess small intestine brush border membrane vesicles (BBMV) function. These vesicles were used to assay the activities of a range of enzymes from the small intestine of these animals (Table 4.48).

Activities of the enzymes assayed in this study were used as an indication of the relative enrichment in enzymatic activity and the influence of a particular diet and/or ingredient on the biochemical processes associated with nutrient uptake and digestion.

Activities of alkaline phosphatase in the BBMV were higher in the soybean and vetch meal fed abalone, though notably the activity of alkaline phosphatase from the *L. luteus* whole-seed meal fed abalone were not as low as those fed either pea or faba bean meals (Table 4.48). Similarly, activities of the BBMV acid phosphatases were also highest in the vetch and soybean meal fed abalone, though activity of the acid phosphatase from the *L. luteus* whole-seed meal fed abalone BBMV was the lowest of all treatments.

Activities of the carbohydrase enzymes (maltase and β-galactosidase) in the BBMV from each of the treatments were only partially consistent with the observations of the phosphatase enzymes (Table 4.48). Maltase activity from BBMV was highest in abalone fed the soybean meal, though these activities were similar to those observed from abalone fed either the *L. luteus* whole-seed meal or pea meal (Table 4.48). The activities of β-galactosidase from BBMV were highest in

soybean meal and lowest in the *L. luteus* whole-seed meal. The β-galactosidase activities were generally consistent with the pattern observed from both the phosphatase enzymes (Table 4.48).

The autoclaving of the protein meals had some affects on the activities of the enzymes assayed in this study. Autoclaving of soybean meal reduced the activity of both phosphatase enzymes. Activity of the maltases, from BBMV of abalone fed the soybean meal diet was not influenced by the autoclaving of the meal, though β-galactosidase were considerably reduced. In contrast, the autoclaving of the *L. luteus* whole-seed meal increased the activity of alkaline phosphatases, but reduced the activity of the acid phosphatase. Activities of both carbohydrase enzymes were increased with the autoclaving of the *L. luteus* whole-seed meal (Table 4.48). Autoclaving of the pea meal improved the activities of both phosphatases and the β-galactosidase, though not the maltase (Table 4.48). Autoclaving of both the vetch and the faba bean meal had little effect on the alkaline phosphatases, though responses of the acid phosphatases were more variable. Maltase activitie were increase in both meals with autoclaving, though the β-galactosidase activities increased in the faba bean meal, but decreased in the vetch (Table 4.48).

In discussing the implications of this study, Kemp et al. (1999) suggested that the elevated enzyme activities were most likely a response aimed at obtaining more nutrients from each respective diet. Few specific comments were made by the authors on what the differences in enzyme activities between the different diets implied. However, these workers did add that they supported the observations of Vandepeer et al. (1999) in suggesting that *L. luteus* whole-seed meal was a suitable ingredient for use in diets for greenlip abalone. Kemp et al. (1999) also suggested that defatted soybean meal was also a useful ingredient in abalone diets. Little support was offered for the use of either vetch or beans as a feed ingredient in the diets of abalone, though suggestions were made that they might be viable options at lower inclusion levels and that this needed further investigation. These workers also suggested that the complementarity between the membrane vesicle studies and the digestibility studies provided good support for use of membrane vesicle as an alternative method of ingredient and diet evaluation. It would have been of value to conduct a more defined comparison of the complementarily between the membrane vesicle studies and the digestibility studies than that presented in the study by Kemp et al. (1999). Determination of correlations between the two study methods would allow a more objective assessment on the value of membrane vesicle studies as a means of assessing ingredients.

Figure 21. Greenlip abalone (Haliotis laevigata)

Table 4.47 *Utilisation of legume meals by the greenlip abalone (Haliotis laevigata). Data derived from Vandepeer et al. (1999).*

	1	2	3	4	5	6	7	8	9	10
	Raw grains					Autoclaved grains				
Diet ingredients (g/kg)										
Defatted soybean meal	333	-	-	-	-	333	-	-	-	-
L. luteus whole-seed meal	-	390	-	-	-	-	390	-	-	-
Field pea meal	-	-	743	-	-	-	-	743	-	-
Vetch meal	-	-	-	638	-	-	-	-	638	-
Faba bean meal	-	-	-	-	664	-	-	-	-	664
Kaolin	200	200	200	200	200	200	200	200	200	200
Pregelled starch	432	375	222	127	101	432	375	222	127	101
Remains	35	35	35	35	35	35	35	35	35	35
Diet proximate composition										
Crude protein (g/kg DM)	179	175	183	180	175	173	171	184	174	174
Crude fat (g/kg DM)	10	20	20	10	13	12	28	31	21	29
Ash (g/kg DM)	235	218	227	231	227	225	219	312	223	218
Crude fibre (g/kg DM)	17	68	60	42	59	3	65	43	39	64
Lysine (g/kg DM)	99	72	111	93	92	83	59	105	88	94
Methionine (g/kg DM)	32	11	13	11	18	25	13	18	21	13
Gross energy (MJ/kg DM)	14.61	14.91	14.84	14.70	14.88	14.59	15.02	18.56	14.69	14.72
Apparent Digestibilities										
Nitrogen / Protein	87	91	75	75	85	66	68	69	69	71
Energy	84	83	49	45	65	68	63	60	56	60
Dry matter	57	61	25	29	42	58	54	57	34	44
Lysine	91	92	80	76	88	63	56	70	68	71
Methionine	92	87	68	55	89	76	59	53	75	68
Phosphorus	86	84	94	85	93	84	78	88	73	77

Table 4.48 Utilisation of legume meals by the greenlip abalone (Haliotis laevigata). Data derived from Kemp et al. (1999).

	1	2	3	4	5	6	7	8	9	10
	Raw grains					Autoclaved grains				
Diet ingredients (g/kg)										
Defatted soybean meal	333	-	-	-	-	333	-	-	-	-
L. luteus whole-seed meal	-	390	-	-	-	-	390	-	-	-
Field pea meal	-	-	743	-	-	-	-	743	-	-
Vetch meal	-	-	-	638	-	-	-	-	638	-
Faba bean meal	-	-	-	-	664	-	-	-	-	664
Kaolin	200	200	200	200	200	200	200	200	200	200
Pregelled starch	432	375	222	127	101	432	375	222	127	101
Remains (unform across treatments)	35	35	35	35	35	35	35	35	35	35
Diet proximate composition	See table 4.47									
*Enzyme activities**										
Alkaline phosphatase (μmol / mg protein / h)	6.7	5.0	4.5	6.5	4.6	4.6	6.0	6.1	6.7	6.5
Acid phosphatase (μmol / mg protein / h)	2.7	0.6	1.0	3.4	1.2	1.0	0.4	2.5	0.7	3.0
Maltase	5.0	4.8	4.8	3.6	2.8	5.0	6.1	4.6	4.8	3.7
β-Galactosidase	1.5	0	0.6	2.0	0.6	0.3	0.6	2.0	0.4	2.2

* Figures are approximations only, determined from interpretation of graphical data.

5.1 Lupins in aquafeed processing

In practical considerations, the nutritional value of lupins plays only part of the importance in whether they are used in commercial diets, and to what extent. The influence that lupins play in the physical characteristics of aquaculture diets is also an important feature though it has not been a prominent feature of research to date. There are, however, some studies where the effects on the physical characteristics of pellets have been examined using both pellet press and extrusion technology.

Traditionally fish and crustacean feeds were made using either screw or pellet press machines. In some instances this included the addition of steam. Generally with these processing technologies, inclusion of fat levels greater that 15% of the diet were not practical. Similarly, the loss of fines and rates of leaching losses from such pellets were often high (Tacon, 1990).

Modern feeds, particularly those fed in intensive fin-fish culture, are usually an extruded product. These feeds typically have a high degree of starch gelatinisation, can hold fat levels greater than 30% of the diet, have fines losses usually less than 1% and similar low levels of leaching losses. However, the processes involved in the preparation of such feeds are typically more costly and complicated.

A study on the nutritional value of several varieties and processing forms of lupins in diets for the giant tiger prawn, *P. monodon*, by Sudaryono et al. (1999a) also examined the pelleting characteristics and water stability of the diets. The treatments evaluated included *L. albus* whole-seed and kernel meals, soybean meal, *L. angustifolius* kernel meal and a lupin protein concentrate (see Table 4.41 and section 4.9 for details on the nutritional and biological performance of these diets).

In this experiment, the most stable pellet, with the least dry matter loss, was observed from the diet with the inclusion of lupin protein concentrate, followed by that using the soybean meal and the two lupin kernel meals. The least stable diet was that which included the *L. albus* whole-seed meal. There were no significant differences in leaching losses between *L. angustifolius* and *L. albus* kernel (Table 5.1).

Complicating the interpretation of these observations though is the concomitant change in the inclusion levels of other ingredients in the diets. Notably, changes in the levels of wheat flour, rice bran and fish oil were made to allow nutrient balancing of the diets in addition to the use of the various lupin and soybean meals.

This study provided a basic account of the potential differences obtained in the water stability of the pellets encountered with the dehulling of the *L. albus* seeds. This observation is of note, in that the same process has also reported an improvement to the nutritional attributes of lupins to most species where the whole-seed and kernel meals have been compared. Of interest though is that the lupin protein concentrate gave the best water stability characteristics. It

would be of value to further define the binding characteristics of lupin meals in diets for prawns, to more fully understand why the removal of the NSP component of the meal improves these properties.

In a second study by the same workers (Sudaryono et al*.,* 1999b), the influence of the serial dilution of the soybean component of prawn diets with *L. albus* kernel meal was evaluated (see Table 4.42 and section 4.9 for details on the nutritional and biological performance of these diets). In this study, a minor improvement in the water stability characteristics of the pellets was observed with the inclusion of *L. albus* kernel meal up to a 75% replacement of the soybean meal component of the diet. With 100% replacement of the soybean meal in the diet with *L. albus* kernel meal a substantial deterioration in the water stability of the pellets was observed (Table 5.2).

As with the earlier study by this group (Sudaryono et al., 1999a) the addition of other key ingredients to the diet when lupins were used to replace the soybean meal complicates the interpretation of this study. Notably, details on particle sizes of each of the meals were also not indicated. Although clear evidence on the influence of particle size on pellet stability is lacking, anecdotal observations (B. Glencross, unpublished) have suggested that this may also be an important factor in pellet stability of pellet-pressed shrimp diets. Other notable points include that changes with increasing *L. albus* kernel meal content were concomitant with an increase in wheat flour content and a decrease in rice bran content of the experimental diets in this study.

Few studies have been conducted where lupins have been examined as a component of extruded aquaculture diets. As part of Australia's Fisheries Research and Development Corporations Fishmeal Replacement Subprogram, a series of studies were undertaken by Gleeson et al. (1998a). These studies compared the properties of soybean meal, a *L. angustifolius* (cv. Gungurru) protein concentrate, and a field pea (*Pisum sativum*, cv. Dun) protein concentrate to a fishmeal reference diet (Table 5.3), when processed as Atlantic salmon feeds through a twin-screw extrusion system (Pilot scale - APV MFP40, APV-Baker, Peterborough, England), (see Table 4.15 and section 4.1.2 for details on the nutritional and biological performance of these diets). To minimise complications associated through the different inclusion levels of ingredients in the diets and variable nutrient levels, the diets were formulated to each provide about 400 g/kg of crude protein and 220 g/kg of crude fat, whilst including each of the plant protein resources at about 280 g/kg (273 to 292 g/kg). Starch levels present in the diets were also kept constant at about 23.2 g/kg.

Table 5.1 *Water stability characteristics of diets formulated for by the prawn, Penaeus monodon, containing substantial levels of soybean, L. albus whole-seed and kernel meals and L. angustfolius kernel meal and lupin protein concentrate. For corresponding nutritional performance of prawns fed the diets see section 4.9, and Table 4.41. Data derived from Sudaryono et al. (1999a).*

	Soybean	*L. albus*		*L. angustifolius*	
		whole-	kernel	kernel	concentrate
Diet ingredients (g/kg)					
Soybean meal	300	-	-	-	-
L. albus whole-seed meal	-	400	-	-	-
L. albus kernel meal	-	-	350	-	-
L. angustifolius kernel meal	-	-	-	360	-
Lupin protein concentrate	-	-	-	-	240
Wheat flour	215	185	235	225	285
Rice bran	50	-	-	-	50
Fish oil	20	-	-	-	10
Fish meal	240	240	240	240	240
Squid meal	50	50	50	50	50
Shrimp meal	70	70	70	70	70
Dicalcium phosphate	10	10	10	10	10
Vitamin premix	10	10	10	10	10
Carboxymethylcellulose	20	20	20	20	20
Soy lecithin	10	10	10	10	10
Chromic oxide	5	5	5	5	5
Period of water immersion	*Percent of dry matter retained*				
1 hour	86.4	78.5	83.2	83.5	88.7
2 hours	83.4	76.2	80.0	79.9	86.2
4 hours	82.0	75.4	78.9	78.3	84.3
8 hours	80.4	73.8	77.3	76.7	83.4

Table 5.2 *Water stability characteristics of diets formulated for the prawn, Penaues monodon examining replacement of soybean meal with L. albus kernel meal. For the corresponding nutritional performance of prawns fed the diets see section 4.9, and Table 4.42. Data derived from Sudaryono et al. (1999b).*

	Soybean meal replacements level (%)				
	0	25	50	75	100
Diet ingredients (g/kg)					
Soybean meal	300	225	150	75	0
L. albus kernel meal	0	90	170	260	350
Wheat flour	185	200	225	230	225
Rice bran	90	60	30	10	0
Fishmeal	240	240	240	240	240
Squid meal	50	50	50	50	50
Shrimp meal	70	70	70	70	70
Fish oil	10	10	10	10	10
Dicalcium phosphate	10	10	10	10	10
Soy lecithin	10	10	10	10	10
Vitamin and mineral premix	10	10	10	10	10
Carboxymethylcellulose	20	20	20	20	20
Chromic oxide	5	5	5	5	5
Period of water immersion	*Percent of dry matter retained*				
1 hour	81.6	82.1	84.6	82.3	79.1
2 hours	79.4	80.0	82.7	81.5	76.2
4 hours	78.2	78.6	82.0	81.1	75.7
8 hours	76.3	76.6	79.7	79.5	74.1

Table 5.3 *Formulations and proximate composition of test diets, processed through feed extrusion, evaluating the processing influences of plant protein resource inclusion. Data derived from Gleeson et al. (1998a).*

	Reference	Soybean	*L. angustifolius* PC	Pea PC
Ingredients				
Fishmeal	601.5	400.0	400.0	400.0
Soybean meal (defatted)	-	272.9	-	-
L. angustifolius protein concentrate	-	-	291.9	-
Pea protein concentrate	-	-	-	275.7
Methionine	-	5.0	6.0	6.0
Fish oil	154.6	159.2	156.4	168.9
Vitamin and mineral premix	7.5	7.5	7.5	7.5
Bentonite	48.2	-	-	-
Cellulose	50.0	17.2	-	26.7
Wheat flour	115.0	115.0	115.0	115.0
Wheat starch	23.2	23.2	23.2	0.2
Diet proximate composition				
Crude Protein (g/kg DM)	410	410	410	410
Crude Fat (g/kg DM)	220	220	220	220
Spray on oil (g/kg DM)	154.6	159.2	156.4	168.9
Total starch (g/kg DM)	105	105	105	105

Table 5.4 *Pellet characteristics of Atlantic salmon diets including plant protein resources, extruded at variable temperatures and screw speeds. Data derived from Gleeson et al. (1998a).*

Pellet characteristics	Reference	Soybean	*L. angustifolius* PC	Pea PC
Temperature: 66℃-82℃, Screw Speed: 200 rpm				
Radial expansion (%)	15.7	28.4	7.7	19.2
Oil absorption (%)	33.2	27.0	15.4	25.2
Bulk density (g/L)	556.6	622.1	735.3	638.2
Sinking rate (cm/s)	0.0	0.5	8.2	4.6
Durability (% loss)	0.5	0.3	0.9	3.0
Shear strength (N/mm^2)	1.6	1.2	1.5	1.2
Hardness (N/mm^2)	9.7	13.5	17.3	19.0
Starch gelatinisation (%)	89.4	97.4	40.1	71.5
Temperature: 88℃-93℃, Screw Speed: 250 rpm				
Radial expansion (%)	21.4	29.3	25.7	29.6
Oil absorption (%)	39.1	28.8	21.6	24.1
Bulk density (g/L)	520.2	611.3	704.9	646.8
Sinking rate (cm/s)	0.0	1.7	4.4	4.5
Durability (% loss)	0.5	0.6	0.6	0.4
Shear strength (N/mm^2)	1.0	1.3	1.3	1.2
Hardness (N/mm^2)	8.3	17.7	16.7	15.5
Starch gelatinisation (%)	90.6	89.0	96.7	91.3
Temperature: 137℃-149℃, Screw Speed: 350 rpm				
Radial expansion (%)	19.5	30.6	30.1	28.9
Oil absorption (%)	38.2	28.9	22.0	27.5
Bulk density (g/L)	496.2	596.7	678.2	582.2
Sinking rate (cm/s)	2.5	4.5	4.4	1.3
Durability (% loss)	3.3	2.8	0.8	1.5
Shear strength (N/mm^2)	0.9	1.4	1.5	1.5
Hardness (N/mm^2)	9.4	23.5	22.8	22.4
Starch gelatinisation (%)	83.6	87.3	91.5	84.8

A range of pellet characteristics of these feeds was modeled using response-surface methodologies, under a range of processing variables. The key processing variables included screw speed and product temperature, though notably residence time of the mash in the extrusion barrel also varies as a function of these two key parameters. The pellet characteristics examined included radial expansion, bulk density, oil absorption, sinking rates, durability, shear strength, hardness and also the degree of starch gelatinisation (Table 5.4).

Each of the pellet characteristics were assessed in specific tests (Evans, 1998). Radial expansion was assessed by the relative change in pellet diameter observed between the die pore diameter and the final pellet diameter. Bulk density was determined on a weight by volume basis. The oil absorption capacity of the pellets was assessed by measuring the weight of oil uptake before and after excess oil addition. The total capacity then being expressed as a sum of the uptake and original oil present in the pellets. The sink rates of the pellets were determined by the time taken for a pellet to sink a defined distance through water. The durability of the pellets was assessed by measuring the losses obtained from tumbling a defined weight of pellets in a dust tight barrel at 50 rpm, for ten minutes, after which the fines were removed with a further sieving for two minutes and re-weighing. The lateral shear strength and hardness of the pellets were determined by measuring compressional force required to either split or crush the pellet, according to specific blades used on a TA-XT2 texture analyser (Arrow Scientific Pty., Ltd., Leichhardt, NSW, Australia). Shear strength of the pellets was defined as the peak force at breaking. Hardness of the pellet was defined as the peak force at breaking, divided by the distance the blade had to move from point of first contact with the pellet till it broke. The degree of starch gelatinisation achieved within the pellets following the extrusion process was assessed using enzymatic methods as described by Evans (1998).

The three plant protein resources tested in this study had quite different attributes in the way they responded to processing as key ingredients in extruded Atlantic salmon feeds.

The greatest degree of radial expansion was observed with the use of soybean meal, though this was not substantially different from that observed of the pea PC. Greater expansion was observed at higher temperatures and with faster screw speeds. Minimal radial expansion was observed of the *L. angustifolius* PC diet at low temperatures and slow screw speeds, though at higher temperatures and faster screw speeds the amount of radial expansion of the pellets was similar to that of the other plant protien treatments and considerably more than that of the reference fishmeal based diet (Table 5.4).

Oil absorption was influenced both by processing conditions and ingredient use (Table 5.4). Generally, oil absorption of the soybean meal diets was influenced by production temperature, but not screw speed. In contrast the oil absorption of the *L. angustifolius* PC diets was influenced by both production temperature and screw speed. The greatest degree of oil absorption was

observed with the use of soybean meal, though this was similar to the level of oil absorption observed of the pea PC (Table 5.4). Greater oil absorption was also observed at higher temperatures and faster screw speeds (Table 5.4). Similar to the observations with radial expansion, minimal oil absorption was observed of the *L. angustifolius* PC diet at low temperatures and slow screw speeds. Although at higher temperatures and faster screw speeds, the amount of oil absorbed by the pellets was similar to that of the other plant protein treatments. Notably, all three plant protein resources has oil absorption levels considerably less than that of the reference fishmeal based diet (Table 5.4).

Bulk density was highest in pellets that were made at the lower temperatures and screw speeds. Typically, the *L. angustifolius* PC diets had a higher bulk density that that of both the soybean and pea PC based diets (Table 5.4). The lowest bulk density, under any of the processing conditions, was that with the reference fishmeal based diet.

Sinking rates of the pellets were influenced primarily by bulk density, with the densest pellets also being those with the fastest sinking rates. However, those pellets with the lowest bulk density (high temperature and screw speed produced reference fishmeal based diet), were not necessarily the pellets with the slowest sink rates (Table 5.4). Pellets of the reference fishmeal based diet, made at low temperatures and screw speeds produced floating pellets. None of the plant protein resource diets resulted in floating pellets, under any of the processing conditions reported. However, the soybean meal based diets produced consistently slower sinking pellets than either pea PC, or the *L. angustifolius* PC diets which were consistently faster sinking.

Pellets made of the *L. angustifolius* PC diet overall had the best durability of the three plant protein resources and reference diets tested (Table 5.4). The greatest variability in the durability of the pellets across the processing conditions was observed from the reference fishmeal based diet. Generally, at the lower temperature and slower screw speed processing conditions, pellets had better durability (Table 5.4). Pea PC diets contrasted this trend though with an improvement in pellet durability with temperature and screw speed increases, with peak performance observed in the mid-range of processing conditions examined.

Pellet shear strength was generally unaffected by either processing conditions or ingredient use. Shear strength of the pellets was however, greatest in pellets of the reference fishmeal based diet processed at the lowest temperature and screw speeds. Pellets made of the *L. angustifolius* PC diets had consistently higher shear strength across the range of processing conditions examined, marginally better than that observed of both the soybean meal and pea PC based diets (Table 5.4).

Pellet hardness was more influenced by processing conditions than ingredient use (Table 5.4). However, little change in pellet hardness was observed in the reference fishmeal based diet with variable processing conditions (8.3 to 9.7 N/mm^2). The greatest

change in pellet hardness with increased processing temperature and screw speed was observed of the soybean meal based diet, though this was not much different from those effects observed in both the *L. angustifolius* PC and pea PC diets (Table 5.4).

Starch gelatinisation was influenced by both processing and ingredient use. Poorest gelatinisation was observed in the *L. angustifolius* PC diet when processed at the lower temperatures and screw speeds (Table 5.4). The highest degree of gelatinisation was observed of the soybean meal diet when also processed at the lower temperatures and screw speeds, though this was not much different from the degree of gelatinisation observed of the *L. angustifolius* PC diet when processed in the mid range of processing conditions examined. In most diets, improvements in the degree of starch gelatinisation where observed with moderate use of temperature (88°C to 93°C) and moderate screw speeds.

A series of correlation matrices were also determined for each of the diets, under the variable processing conditions. The key finding for the soybean meal based diets only, was the high level of correlation ($r^2 > 0.8$) between oil absorption and bulk density. Few other relationships were evident between the various physical characteristics of the soybean meal based diets.

The *L. angustifolius* PC diet had considerably more correlations among the pellet characteristics than that observed for the soybean meal based diets. Key correlations of note ($r^2 > 0.8$) were observed between, radial expansion and starch gelatinisation, oil absorption and starch gelatinisation, sinking rate and bulk density, durability and starch gelatinisation and the shear strength and hardness of the pellet.

This study presents a valuable account of the influence of three plant protein resources on the characteristics of pellets produced through extrusion pelleting. Clearly the value of this study is the identification of the flexibility of the feed producer to be able to tailor diets of a defined nutrient composition to a range of desired physical attributes. This study in this essence demonstrating that this can be achieved through control of both ingredient choice and processing conditions.

An additional study by these same workers (Gleeson et al., 1998b), also examined the extrusion processing characteristics of diets for silver perch, based on the formulations by Allan et al. (2000). In two of the formulations included in this processing study, *L. angustifolius* kernel meals were included. One diet containing *L. angustifolius* kernel meal at 255 g/kg and the other at 73 g/kg. All diets were formulated to the same digestible protein and energy contents. However, in comparison to the study examining the processing characteristics of the diets for Atlantic salmon (Gleeson et al., 1998a), the processing characteristics of the diets formulated for the silver perch were more difficult to attribute to specific ingredients, because of the wide range of ingredients used in each diet, and the lack of a standardised control. Accordingly, the specific details of this study are not reviewed here, despite the use of *L. angustifolius* kernel meal in the diets.

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