



A digestibility assessment of pearl lupin (*Lupinus mutabilis*) meals and protein concentrates when fed to rainbow trout (*Oncorhynchus mykiss*)

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ABSTRACT

Two experiments were undertaken to examine the digestible value of a range of grain products produced from seed of the pearl lupin *Lupinus mutabilis*. These products were then evaluated against similar products produced from the lupin species *L. angustifolius* and *L. luteus* and solvent-extracted soybean meals. The value-added products were then included in diets at a 300 g/kg inclusion level to assess their apparent dry matter, protein and energy digestibilities. It was observed that *L. mutabilis* kernel meals had superior digestible value characteristics to that of *L. angustifolius* kernel meal and similar values to that of solvent-extracted soybean and *L. luteus* kernel meals. The use of solvent-extractive value-adding techniques only marginally increased the protein content of *L. mutabilis* kernel meal, but reduced their digestible energy value. A more significant increase in protein content was observed using aqueous extractive techniques, although this had mixed effects on the digestible value of the products. The use of protein isolation techniques substantially increased the protein content and digestible value of products produced from each of the species examined. This work demonstrates that grain from *L. mutabilis* seed can be effectively processed to produce meals that have digestible values potentially useful for aquaculture feeds.

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1. Introduction

The use of grain products in aquaculture feeds is now common-place in the diet formulations of many species (Gatlin et al., 2007). Among those grain raw materials frequently being used are lupins (Glencross et al., 2005, 2006a,b, 2007a). There are several species of lupins that have been evaluated and/or used as feed raw materials; these include *Lupinus albus*, *L. angustifolius* and *L. luteus* (Burel et al., 2000; Glencross and Hawkins, 2004; Refstie et al., 2006). However, there are no reports on the nutritional value of the pearl lupin, *Lupinus mutabilis* when fed to fish.

The pearl lupin, *L. mutabilis*, is a species native to the Andean areas of South America. Efforts are being made in Australia to domesticate this species by reducing its alkaloid levels and improving key agronomic characteristics (Sweetingham et al., 2006; Clements et al., 2008). At present there is no published data on its nutritional value when fed to any domestic animal species.

In addition to domestication of the grain, the high protein and high lipid characteristics of this grain mean that it is potentially suitable for further processing for its protein and oil fractions. There are also a range of options that can be used to improve the nutritional value of lupins and

typically whole-seed lupins are not used in aquaculture feeds. Generally dehulled kernel meal proves to be the most cost-effective protein-enriched form of the grain (Glencross et al., 2007b). Dehulling has also been shown to be the first step of improving the nutritional value of lupins in regard to the development of protein concentrates and isolates and in this form has shown significant improvements in nutritional value of the grain as a feed ingredient (Glencross et al., 2006a).

Therefore the present study begins this evaluation with an examination of the digestibility of a series of products produced from *L. mutabilis* seed when fed to rainbow trout (*Oncorhynchus mykiss*). In a series of two experiments the products were examined; Experiment 1—protein concentrate and isolate products and Experiment 2—full-fat and defatted kernel meals. These products were also benchmarked against other key lupin and soybean products to provide a comparative evaluation of their nutritional value.

2. Materials and methods

2.1. Ingredient development

Two samples of the *L. mutabilis* seed were obtained from the Department of Agriculture and Food, Western Australia's International Lupin Collection, dehulled and milled to create stock samples *L. mutabilis* kernel meal. Dehulling was conducted as described in Glencross et al. (2007b). After dehulling the kernels were milled through a 750 µm

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screen on a rotor mill (Retsch, Haan, Germany). The 2004 sample (accession P26961) was a high alkaloid line (>2000 mg/kg) and was used to prepare both protein isolates and concentrates. The 2005 sample (breeding line ID13) which was low in alkaloids (<200 mg/kg) was used to prepare kernel meals and were also defatted and kept raw to produce both full-fat and defatted kernel meals. Samples of *L. angustifolius* cv Myallie and *L. luteus* cv Wodjil kernel meals were obtained from a commercial grain processor and concentrates and isolates prepared as detailed in Glencross et al. (2006a) and below.

The defatted *L. mutabilis* kernel meal was produced using hexane extraction of a kernel meal sample. Three volumes of hexane to kernel meal were mixed in an upright mixer using a K-blade (Hobart, Sydney, Australia). The mixture was then filtered through a piece of muslin cloth to remove the hexane, before it was oven dried at 60 °C for 12 h. The dried defatted lupin meal was re-milled to ensure all particles were <750 µm particle size.

Protein concentrates (PC) from *L. angustifolius*, *L. luteus* and *L. mutabilis* were prepared from cooked flours (autoclaved) at 122 °C for 60 min (inclusive of ramp up, ramp down and depressurisation). Following cooking the flours were sieved through a 3000 µm sieve and water added to produce a 15:1 mix of water to flour. This mix was stirred for 60 min before being filtered through an 800 µm filter bag. The liquid from each wash after being filtered through the 800 µm bag was then filtered through a 50 µm bag and that residue was added to the rest of the residue that had been left from the first filtration. The residue was then washed again in water (15:1, water:residue) for 20 min before being filtered for a second time through an 800 µm and 50 µm filter bags as previously. The *L. mutabilis* sample had 2 further 5 min washes that included filtration through an 800 µm and 50 µm filter bags each time after washing. Then all residues were frozen at –20 °C prior to being freeze-dried. Following the freeze-drying process, each of the PC's was re-milled to ensure all particles were <750 µm particle size.

Protein isolates from *L. angustifolius* and *L. luteus* were prepared from samples of each meal that were solubilised in water at room temperature and the pH adjusted to 9.0 with NaOH (1.0 M) with vigorous stirring for 60 min. After mixing, the solution was filtered through an 800 µm filter bag to separate the non-solubilised material from the solubilised protein. The protein solution was then brought to a pH of 4.5 with the addition of HCl (2.0 M) at room temp and allowed to precipitate out of solution whilst held at 4 °C. The protein precipitate was decanted after 12 h and dried in a freeze drier. The extraction processes are based on those reported in Laszity et al. (2001). Following the freeze-drying process, both of the PI's was re-milled to ensure all

particles passed through a 750 µm rotor mill screen. Insufficient *L. mutabilis* kernel meal was available to produce a protein isolate.

The composition and source of all of the ingredients used are presented in Table 1. Each of the test ingredients was thoroughly ground such that they passed through a <800 µm particle size screen.

2.2. Diet development

The experiment design was based on a diet formulation strategy that allowed for the diet-substitution digestibility method to be used. For this, a basal diet was formulated and prepared to include approximately 500 g/kg DM protein, 210 g/kg DM fat and an inert marker (yttrium oxide at 1 g/kg) (Tables 2 and 3). A basal mash was prepared and thoroughly mixed, forming the basis for all experimental diets in this study. The ingredient of study for each test diet was added at 30% inclusion to a sub-sample of the basal mash. Diets were processed by addition of water (about 30% of mash dry weight) to the mash whilst mixing to form a dough, which was subsequently screw pressed using a pasta maker through a 4 mm diameter die. The resultant moist pellets were then oven dried at 70 °C for approximately 12 h and then allowed to cool to ambient temperature in the oven. The basal diet was prepared in a similar manner, but without the addition of any test ingredient. Two independent experiments were undertaken to evaluate the products produced from different years. The diet formulations and source of all of the ingredients used is presented in Tables 2 and 3. The composition of diets from Experiment 1 is presented in Table 2 and those from Experiment 2 are presented in Table 3.

2.3. Fish handling and faecal collection

Two independent experiments were conducted using hatchery-reared rainbow trout (*O. mykiss*, Pemberton heat-tolerant strain, Western Australia). Conditions for each experiment were: Experiment 1—Freshwater (salinity <1 PSU) of 16.1 ± 0.3 °C (mean ± S.D.), dissolved oxygen 8.5 ± 0.3 mg/L. Each of the experimental tanks (200 L) were stocked with 15 trout of 361 ± 43.7 g (mean ± S.D.; n = 40 sample). Experiment 2—Freshwater (salinity <1 PSU) of 16.3 ± 0.2 °C, dissolved oxygen 8.0 ± 0.6 mg/L. Tanks were stocked with 15 trout of 210 ± 16.7 g (mean ± S.D.; n = 40 sample). Treatments were randomly assigned amongst the tanks, with each treatment having three replicates. Water flow rate was maintained at about 4 L/min to each of the tanks.

Fish were manually fed the diets once daily to apparent satiety as determined over three separate feeding events between 1500 and

Table 1
Nutrient composition of the experimental ingredients (all values are g/kg DM unless otherwise indicated).

Nutrient	^a Fish meal	^a Wheat	Soybean meal	AKM	APC	API	LKM	LPC	LPI	MKM	DMKM	MPC
Dry matter content (g/kg)	931	905	899	916	918	937	909	939	931	924	933	939
Crude protein	749	142	497	412	500	754	537	719	819	515	561	759
Crude fat	87	24	21	97	69	153	77	62	112	171	66	87
Ash	161	11	75	35	13	23	44	14	29	61	66	13
Phosphorus	28	2	8	5	3	7	7	3	7	6	7	3
Gross energy (MJ/kg DM)	20.5	18.4	19.9	20.6	20.7	25.1	21.1	22	24.2	23.0	20.5	23.1
Arginine	39	7	35	43	48	84	53	66	83	49	53	65
Cysteine	9	4	9	7	8	12	16	20	24	9	10	16
Histidine	18	1	12	9	12	17	14	17	18	13	14	20
Isoleucine	33	5	23	16	24	35	21	29	31	20	22	34
Leucine	60	10	42	29	43	64	44	64	72	32	35	61
Lysine	51	5	32	13	22	33	27	27	36	19	21	31
Methionine	26	2	9	4	5	6	5	8	7	3	3	8
Phenylalanine	30	6	27	16	23	34	22	29	33	17	19	29
Threonine	37	5	24	17	23	32	19	30	29	18	20	35
Valine	39	6	23	14	20	29	17	25	26	17	19	28

^a Wheat and Fish meal: Chilean anchovy meal, Skretting Australia, Cambridge, TAS, Australia. SBM: Solvent-extracted soybean meal: WESFEEDS, Bentley, WA, Australia. AKM: *L. angustifolius* kernel meal: Coorow Seed Cleaners, Coorow, WA, Australia. APC: *L. angustifolius* protein concentrate, API: *L. angustifolius* protein isolate. LKM: *L. luteus* kernel meal. LPC: *L. luteus* protein concentrate. LPI: *L. luteus* protein isolate. MKM: *L. mutabilis* kernel meal. DMKM: Solvent-extracted *L. mutabilis* kernel meal. MPC: *L. mutabilis* protein concentrate: Department of Agriculture and Food—Government of Western Australia, South Perth, WA, Australia.

Table 2

Formulations of the diets for experiment 1 (all values are g/kg).

	Reference Diet	SBM	AKM	APC	API	LPC	LPI	MPC
<i>Ingredient</i>								
Fishmeal	700.0	490.0	490.0	490.0	490.0	490.0	490.0	490.0
Fish oil	150.0	105.0	105.0	105.0	105.0	105.0	105.0	105.0
Solvent-Extracted Soybean meal		300.0						
<i>L. angustifolius</i> kernel meal			300.0					
<i>L. angustifolius</i> concentrate				300.0				
<i>L. angustifolius</i> isolate					300.0			
<i>L. luteus</i> concentrate						300.0		
<i>L. luteus</i> isolate							300.0	
<i>L. mutabilis</i> concentrate								300.0
Wheat flour	144.0	100.8	100.8	100.8	100.8	100.8	100.8	100.8
Vitamin and mineral premix*	5.0	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Yttrium oxide	1.0	0.7	0.7	0.7	0.7	0.7	0.7	0.7
<i>Diet composition as analysed</i>								
Dry matter	961	964	952	949	962	946	960	958
Protein	494	498	478	504	575	565	586	574
Fat	233	172	186	185	195	182	179	190
Carbohydrate**	149	222	239	221	139	163	142	146
Phosphorus	19	15	14	14	15	14	15	14
Ash	124	108	97	90	90	90	93	90
Gross Energy (MJ/kg)	22.9	21.7	22.4	22.4	23.4	22.8	23.0	23.0

SBM: Solvent-extracted soybean meal, AKM: *L. angustifolius* kernel meal, APC: *L. angustifolius* protein concentrate, API: *L. angustifolius* protein isolate, LPC: *L. luteus* protein concentrate, LPI: *L. luteus* protein isolate, MPI: *L. mutabilis* protein isolate. *Vitamin and mineral premix includes (IU/kg or g/kg of premix): Vitamin A, 2.5MIU; Vitamin D3, 0.25 MIU; Vitamin E, 16.7 g; Vitamin K3, 1.7 g; Vitamin B1, 2.5 g; Vitamin B2, 4.2 g; Vitamin B3, 25 g; Vitamin B5, 8.3; Vitamin B6, 2.0 g; Vitamin B9, 0.8; Vitamin B12, 0.005 g; Biotin, 0.17 g; Vitamin C, 75 g; Choline, 166.7 g; Inositol, 58.3 g; Ethoxyquin, 20.8 g; Copper, 2.5 g; Ferrous iron, 10.0 g; Magnesium, 16.6 g; Manganese, 15.0 g; Zinc, 25.0 g. **Carbohydrate determined as dry matter minus protein, fat and ash.

1600 each day. The trout were allowed to acclimatise to the allocated dietary treatment for seven days before faecal stripping collection commenced consistent with earlier studies by this group (Glencross et al., 2005). Fish were netted from their respective tank, placed in a smaller aerated tank containing isoeugenol (0.002 mL/L) until they lost consciousness. The faeces were then removed from the distal intestine using gentle abdominal pressure. Care was taken to ensure that the faeces were not contaminated by urine or mucous. After removal of the faeces from the fish, the faecal sample was placed in a

small plastic vial and stored in a freezer at -20°C . Stripped faeces were collected during 0800 to 1000 h over a four-day period, with each fish only being stripped twice and not on consecutive days. Faecal samples from different days were pooled within tank, and kept frozen at -20°C before being freeze-dried in preparation for analysis.

2.4. Chemical and digestibility analysis

All chemical analyses were carried out by NATA (National Association of Testing Authorities) accredited analytical service providers (Chemistry Centre (WA), East Perth, WA, Australia, Animal Health Laboratories, South Perth, WA, Australia and SARDI Pig and Poultry Production Institute, Roseworthy, SA). Diet and faecal samples were analysed for dry matter, yttrium, ash, phosphorus, nitrogen and gross energy content. Dry matter was calculated by gravimetric analysis following oven drying at 105°C for 24 h. Total yttrium and phosphorus concentrations were determined after mixed acid digestion using inductively coupled plasma atomic emission spectrophotometry (ICP-AES) based on the method described by McQuaker et al. (1979). Protein levels were calculated from the determination of total nitrogen by Leco auto-analyser, based on $N \times 6.25$. Amino acid composition of samples was determined by acid hydrolysis prior to separation via HPLC. The acid hydrolysis destroyed tryptophan making it unable to be determined. Crude fat content of the diets was determined gravimetrically following extraction of the lipids according to the method of Folch et al. (1957). Gross ash content was determined gravimetrically following loss of mass after combustion of a sample in a muffle furnace at 550°C for 12 h. Gross energy was determined by adiabatic bomb calorimetry. Differences in the ratios of the parameters of dry matter, protein, amino acids or gross energy to yttrium, in the feed and faeces in each treatment were calculated to determine the apparent digestibility coefficient (ADC_{diet}) for each of the nutritional parameters examined in each diet based on the following formula (Maynard and Loosli, 1979):

$$\text{ADC}_{\text{diet}} = 1 - \left(\frac{Y_{\text{diet}} \times \text{Parameter}_{\text{faeces}}}{Y_{\text{faeces}} \times \text{Parameter}_{\text{diet}}} \right)$$

Table 3

Formulations of the diets for experiment 2 (all values are g/kg).

	Reference Diet	SBM	AKM	MKM	DFMKM
<i>Ingredient</i>					
Fishmeal	700	490	490	490	490
Fish oil	150	105	105	105	105
Solvent-Extracted Soybean meal		300			
<i>L. angustifolius</i> kernel meal			300		
<i>L. mutabilis</i> kernel meal				300	
Solvent-Extracted <i>L. mutabilis</i> kernel meal					300
Wheat flour	144	100.8	100.8	100.8	100.8
Vitamin and mineral premix*	5	3.5	3.5	3.5	3.5
Yttrium oxide	1	0.7	0.7	0.7	0.7
<i>Diet composition as analysed</i>					
Dry matter	954	947	951	956	948
Protein	517	505	450	489	508
Fat	219	165	169	208	179
Carbohydrate**	85	159	332	148	146
Ash	133	118	0	111	114
Gross Energy (MJ/kg)	22.9	22.0	22.1	22.8	22.4

SBM: Solvent-extracted soybean meal, AKM: *L. angustifolius* kernel meal, APC: *L. angustifolius* protein concentrate, API: *L. angustifolius* protein isolate, LPC: *L. luteus* protein concentrate, LPI: *L. luteus* protein isolate, MPI: *L. mutabilis* protein isolate. *Vitamin and mineral premix includes (IU/kg or g/kg of premix): Vitamin A, 2.5MIU; Vitamin D3, 0.25 MIU; Vitamin E, 16.7 g; Vitamin K3, 1.7 g; Vitamin B1, 2.5 g; Vitamin B2, 4.2 g; Vitamin B3, 25 g; Vitamin B5, 8.3; Vitamin B6, 2.0 g; Vitamin B9, 0.8; Vitamin B12, 0.005 g; Biotin, 0.17 g; Vitamin C, 75 g; Choline, 166.7 g; Inositol, 58.3 g; Ethoxyquin, 20.8 g; Copper, 2.5 g; Ferrous iron, 10.0 g; Magnesium, 16.6 g; Manganese, 15.0 g; Zinc, 25.0 g. **Carbohydrate determined as dry matter minus protein, fat and ash.

Table 4
Digestibility (%) specifications for experiment 1 of diets and test ingredients and digestible nutrient content (g/kg DM, unless otherwise detailed) of the test ingredients as determined using stripping faecal/digesta collection methods.

Nutrient	Reference	SBM	AKM	APC	API	LPC	LPI	MPC	Pooled SEM
<i>Diet digestibility</i>									
Dry matter	0.835 ^a	0.775 ^b	0.728 ^c	0.709 ^c	0.847 ^a	0.777 ^b	0.833 ^a	0.818 ^{ab}	0.011
Protein	0.909 ^c	0.910 ^c	0.914 ^c	0.896 ^d	0.933 ^a	0.902 ^d	0.924 ^b	0.918 ^b	0.002
Energy	0.910 ^a	0.854 ^b	0.816 ^c	0.814 ^c	0.909 ^a	0.858 ^b	0.905 ^a	0.891 ^{ab}	0.008
<i>Ingredient digestibility</i>									
Dry matter	–	0.685 ^c	0.478 ^b	0.405 ^a	0.901 ^c	0.620 ^c	0.853 ^d	0.789 ^d	0.021
Protein	–	0.933 ^b	0.992 ^a	0.917 ^c	0.977 ^{ab}	0.903 ^c	0.921 ^c	0.931 ^{bc}	0.007
Energy	–	0.648 ^c	0.595 ^c	0.585 ^c	0.884 ^a	0.762 ^b	0.855 ^a	0.848 ^a	0.019
<i>Digestible nutrients</i>									
Dry matter	–	616	438	372	844	582	794	741	
Protein	–	464	409	458	737	649	754	707	
Energy (MJ/kg DM)	–	12.9	12.3	12.1	22.2	16.8	20.7	19.6	

SBM: Solvent-extracted soybean meal, AKM: *L. angustifolius* kernel meal, APC: *L. angustifolius* protein concentrate, API: *L. angustifolius* protein isolate, LPC: *L. luteus* protein concentrate, LPI: *L. luteus* protein isolate, MPC: *L. mutabilis* protein concentrate. Different superscripts within rows indicate significant differences between means among ingredients, but not between nutrients or Diet/Ingredient assessment ($P < 0.05$). Digestible nutrient values are calculated based on ingredient composition (Table 1) and ingredient apparent digestibility coefficients (Table 3). Where apparent digestibility coefficients were greater than 100%, an absolute digestibility of 100% was assumed for practicality reasons.

Where Y_{diet} and Y_{faeces} represent the yttrium content of the diet and faeces respectively, and $\text{Parameter}_{\text{diet}}$ and $\text{Parameter}_{\text{faeces}}$ represent the nutritional parameter of concern (organic matter, protein or energy) content of the diet and faeces respectively. Digestibility values for each diet are presented in Tables 4 and 5. The digestibility values for each of the test ingredients in the test diets examined in this study were calculated according to the formulae:

$$\text{Nutr.AD}_{\text{ingredient}} = \frac{(\text{AD}_{\text{test}} \times \text{Nutr}_{\text{test}} - (\text{AD}_{\text{basal}} \times \text{Nutr}_{\text{basal}} \times 0.7))}{(0.3 \times \text{Nutr}_{\text{ingredient}})}$$

Where $\text{Nutr.AD}_{\text{ingredient}}$ is the digestibility of a given nutrient from the test ingredient included in the test diet at 30%. AD_{test} is the apparent digestibility of the test diet. AD_{basal} is the apparent digestibility of the basal diet, which makes up 70% of the test diet. $\text{Nutr}_{\text{ingredient}}$, $\text{Nutr}_{\text{test}}$ and $\text{Nutr}_{\text{basal}}$ are the level of the nutrient of interest in the ingredient, test diet and basal diet respectively (Sugiura et al., 1998). All raw material

inclusion levels were also corrected for dry matter contribution and the effects that this may have had on the actual ratio of reference diet to test ingredient (Bureau and Hua, 2006).

2.5. Statistical analysis

All values are means unless otherwise specified. Data were analysed for homogeneity using Cochran's test. Effects of ingredient on digestibility of dry matter, protein and gross energy in each of the ingredient were examined by one-way ANOVA (Tables 4 and 5). Levels of significance were determined using a Least Significant Difference (LSD) test. Limits for all critical ranges were set at $P < 0.05$.

3. Results

3.1. Ingredient composition

The ingredients produced in this study, were from one of three different species of lupin seed and had a range of compositions (Table 1). The *L. mutabilis* kernel meal (MKM), in contrast to the other lupin varieties had a substantially higher lipid content and a protein content similar to *L. luteus* kernel meal (LKM). The defatted *L. mutabilis* kernel meal (DFMKM) had a lower lipid content and a higher protein content than its untreated counterpart. The amino acid composition of *L. mutabilis* kernel meal (MKM) is lower in methionine and cysteine (the sulphur amino acids) than *L. luteus* (LKM) and *L. angustifolius* (AKM) kernel meals.

Only a marginal increase in protein content was observed between the *L. angustifolius* kernel meal (AKM) and protein concentrate (APC) though more significant gains were achieved in protein content through the isolation process (e.g. API) (Table 1). A substantially greater increase in protein content was observed between the *L. mutabilis* and *L. luteus* kernel meals and protein concentrates (MPC and LPC) (Table 1). Protein concentrates were typically lower in crude fat than both the kernel meals and the protein isolates (Table 1).

3.2. Ingredient digestibility—Experiment 1

Apparent dry matter digestibilities of the value-added grain products generally improved with increasing protein content across most grain varieties (Table 4). An exception to this was the dry matter digestibility of the APC, which was lower than that of the AKM. The API also had higher dry matter digestibility than the LPI, despite having lower combined protein and fat levels. The API had the highest

Table 5
Digestibility (%) specifications for experiment 1 of diets and test ingredients and digestible nutrient content (g/kg DM, unless otherwise detailed) of the test ingredients as determined using stripping faecal/digesta collection methods.

	Reference	SBM	AKM	MKM	DFMKM	Pooled SEM
<i>Diet digestibility</i>						
Dry matter	80.9 ^a	76.9 ^b	72.7 ^c	78.1 ^{ab}	75.9 ^{bc}	0.78
Protein	89.7 ^a	91.9 ^a	98.1 ^c	95.5 ^b	94.1 ^b	0.83
Energy	90.5 ^a	84.8 ^{bc}	82.0 ^d	86.9 ^b	84.1 ^c	0.83
<i>Ingredient digestibility</i>						
Dry matter	–	69.1 ^{ab}	54.0 ^c	74.4 ^a	63.6 ^b	2.51
Protein	–	93.1 ^a	96.3 ^b	92.4 ^a	91.5 ^a	0.61
Energy	–	69.0 ^b	58.3 ^c	75.9 ^a	69.8 ^{ab}	2.10
<i>Digestible nutrients</i>						
Dry matter		691	494	687	594	26.9
Protein		463	397	476	514	14.0
Energy (MJ/kg DM)		13.7	12.0	17.4	14.3	0.7

SBM: Solvent-extracted soybean meal, AKM: *L. angustifolius* kernel meal, APC: *L. angustifolius* protein concentrate, API: *L. angustifolius* protein isolate, LPC: *L. luteus* protein concentrate, LPI: *L. luteus* protein isolate, MPC: *L. mutabilis* protein concentrate. Different superscripts within rows indicate significant differences between means among ingredients, but not between nutrients or Diet/Ingredient assessment ($P < 0.05$). Digestible nutrient values are calculated based on ingredient composition (Table 1) and ingredient apparent digestibility coefficients (Table 3). Where apparent digestibility coefficients were greater than 100%, an absolute digestibility of 100% was assumed for practicality reasons.

(90.1) dry matter digestibility of all the products evaluated and the APC the lowest (40.5) (Table 4).

Apparent protein digestibilities of the value-added grain products were largely unaffected by the increased protein content of the value-adding processes (Table 4). Indeed a significant decline in protein digestibility was observed between the AKM and APC. The APC also had a significantly lower protein digestibility than the API, but the same observation was not consistent between the LPC and the LPI. Protein digestibility of the MPC was similar to that of the LPC and LPI, but both were lower than that of the API. The AKM had the highest protein digestibility (99.2) of all products evaluated and the LPC the lowest (90.3) (Table 4).

Apparent energy digestibilities of the value-added grain products were significantly improved by the increased protein content of the value-adding processes (Table 4). Although a significant decline in energy digestibility was observed between the AKM and APC. The APC also had a significantly lower energy digestibility than the API, but the same observation was not as consistent to the same degree between the LPC and the LPI. The energy digestibility of the MPC was similar to that of the LPI, and was higher than that of both the APC and LPC. The API had the highest energy digestibility (88.4) of all products evaluated and the APC the lowest (58.5) (Table 4).

3.3. Ingredient digestibility—Experiment 2

The apparent dry matter digestibilities of the MKM and DFMKM products was significantly higher than that of the AKM and the MKM was significantly higher than the SBM apparent dry matter digestibility. The MKM had the highest (74.4) dry matter digestibility of all the products evaluated and the AKM the lowest (54.6) (Table 5).

Apparent protein digestibilities of the MKM and DFMKM products were not significantly higher than that of the SBM and were both lower than that of the AKM (Table 5). A numerically lower protein digestibility, but not significantly so, was seen on the defatting of the MKM to produce the DFMKM. The AKM had the highest protein digestibility (96.3) of all products evaluated and the DFMKM the lowest (91.5) (Table 5).

The apparent energy digestibility of the MKM was significantly higher than that of the DFMKM, SBM and AKM (Table 5). There were no differences between the energy digestibility of the DFMKM and the SBM. The energy digestibility of the AKM was significantly lower than that of all the other ingredients. The MKM had the highest energy digestibility (75.9) of all products evaluated and the AKM the lowest (58.3) (Table 5).

4. Discussion

Most studies on the nutritional assessment of lupin meals have focussed on products from the *Lupinus angustifolius*, *L. albus* and *L. luteus* species, but this is the first assessment of the *L. mutabilis* species in any form when fed to a fish (Burel et al., 2000; Glencross and Hawkins, 2004; Glencross et al., 2005, 2006a, 2007a). While the advantages of dehulled versus whole-seed lupins have been made clear across a range of fish species (Booth et al., 2001; Glencross et al., 2007b), it is also known that further benefits may be obtained by using products such as protein concentrates and isolates from these lupin species (Glencross et al., 2005, 2007a). As such an attempt was made in this study to examine the nutritive value of *L. mutabilis* meals in a range of processing forms.

4.1. Ingredient composition

The *L. mutabilis* meals used in this study were notable in that they had higher protein than that seen in most other lupin species. In addition to this higher protein the native *L. mutabilis* kernel meal also had a lipid level similar to that seen in full-fat soybean meal (Kaushik

et al., 1995). This combination of high protein and high lipid levels within the *L. mutabilis* kernel meal indicates that this grain has good potential for not only direct application to aquaculture feeds, but also to additional processing to produce oils and protein-enriched meals as is done in the soybean industry.

In addition to the native kernel meals and the solvent-extracted meals, a range of additional products were produced using established concentrate and isolate technologies (Lasztity et al., 2001; Glencross et al., 2005, 2006a). The ingredients produced in this study, were produced from one of three different species of lupin and had a range of compositions consistent with the potential range in protein content observable between lupin kernel meals and protein isolates as reported in other studies (Glencross et al., 2005, 2006a). The protein isolation process as has been observed in other studies was far more successful in concentrating the protein, but it is notably a less efficient process with much lower yields. That only a marginal increase in protein content was observed between the *L. angustifolius* kernel meal (AKM) and protein concentrate (APC) would also raise an important question concerning the cost associated with such an extractive process. Would the cost of value-adding be recouped in the value of the final product? Clearly more significant gains were achieved in protein content through the isolation processes for each of the grain varieties studied and this process also appears to produce a more nutrient dense product. However, the composition of the “ideal” specifications for such a value-added grain product for the aquaculture sector are difficult to define precisely, as they will depend on a variety of factors such as cost and availability of other alternatives and also the cost and efficiency of any value-adding processes used (Glencross, 2003).

It is interesting that the protein concentrates were typically lower in crude fat than both the kernel meal and the protein isolates from their respective lupin species. This supports the idea that the extractive processes used to prepare the concentrates also removed a significant component of the kernel meal lipid. While removal of the lipid can be regarded as a value-adding process through the redirection of the lipid to other uses, as in some sectors such as the soybean industry, in this case however it has substantially reduced the nutritional value of the protein concentrate from a compositional perspective.

4.2. Ingredient digestibilities and nutritional value

The *L. mutabilis* kernel meals in both their native and solvent-extracted forms proved to be ingredients with high nutritive value for use in fish diets. Both meals had significant advantages over *L. angustifolius* kernel meal and the solvent-extracted *L. mutabilis* kernel meal had largely similar characteristics to the solvent-extracted soybean meal to which it was compared. The solvent-extraction of *L. mutabilis* kernel meal marginally, but not significantly reduced the protein digestibility, although the total digestible protein content increased due to the protein concentrating effect of removing the lipid content of the kernel meal. However the digestible energy content of the solvent-extracted meal was substantially reduced through the removal of the lipid fraction. This effect is also similar to other reports comparing the digestible value of full-fat and solvent-extracted soybean meals (Kaushik et al., 1995). Examination of the dry matter digestibilities with the protein digestibilities and assuming that the majority of the lipid is digested also indicates that little of the carbohydrate fraction (mostly non-starch polysaccharides in lupins) is absorbed from either of the *L. mutabilis* meals.

Significant improvements in most digestible parameters were observed with increasing levels of protein concentration of the different lupin species. The key exception to this was the digestible value of the protein concentrates APC, LPC and MPC, which despite increases in their protein content had reduced relative values of that protein and also their energy content. It is suspected that this may have occurred through damage to the nutritional value of the protein in these value-added products when autoclaving the kernel meals

during their manufacture, similar to what was reported in Glencross et al. (2007b,c) from the application of heat in the drying process.

By comparison of the digestible dry matter, protein and energy values it is possible to deduce the nutritional value derived from the various components in each ingredient. For example, the soybean and *L. angustifolius* kernel meal both have similar levels of digestible protein (464 g/kg DM vs 409 g/kg DM), but the soybean has a markedly higher dry matter digestibility (616 g/kg DM vs 438 g/kg DM). This suggests that based on the fact that there is limited lipid in the soybean meal and the similarity of the energy digestibility of the two grains, that substantial amounts of the soybean carbohydrates are absorbed, while those of the lupin kernel meal are not. This observation is consistent with other reports on the digestibility of soybean and lupin kernel meals when fed to trout (Kaushik et al., 1995; Glencross et al., 2005).

Another interesting comparison is that between the AKM and the APC (Table 4). Given that the APC is derived from the extractive processing of the AKM, it can be noted that there is a net decline in the digestible dry matter, protein and energy of the APC. The AKM had a digestible protein level of 409 g/kg DM, while the APC had a digestible protein level of 372 g/kg DM. The digestible energy declined from 12.3 to 12.1 MJ/kg DM also. This supports that the processes used to produce the APC have in fact deteriorated its nutritional value as a feed product for fish. Possible reasons for this may be that much of the protein has been damaged, reducing its digestible value (Glencross et al., 2007c), or that the processing has changed the nutritional profile of what is there, that is to increase the level of fibre in the ingredient as has been observed in other studies (Glencross et al., 2007c, 2007a).

A comparison among the protein isolates produced from each lupin variety (API and LPI) show that irrespective of starting material, this value-adding process consistently produces products of the highest protein content and most consistent digestible value (Tables 1 and 4). The process also retains significant amounts of lipid. However, the high digestible protein content probably exceeds that needed for a bulk-commodity required to replace substantial amounts of fish meal in aquaculture diets.

4.3. Conclusions

This study shows that there are some compositional and nutritional features of *L. mutabilis* that make it an interesting prospective protein source for application to aquaculture feeds. The processing of the grain species to produce kernel meals, defatted kernel meals and protein concentrates shows that this grain species has many of the positive attributes shared with other lupin species. Indeed, its high protein and lipid levels make it highly suitable for the application to modern fish diets. Although the use of *L. mutabilis* as a new grain species for use in aquaculture feeds shows substantial promise, further assessment of its nutritional value in growth studies would be appropriate.

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