This is the peer reviewed version of the following article: Glencross, B., Bourne, N., Hawkins, W., Karopoulos, M., Evans, D., Rutherford, N., McCafferty, P., Dods, K., Burridge, P., Veitch, C., Sipsas, S., Buirchell, B. and Sweetingham, M. (2015), Using Near Infrared Reflectance Spectroscopy (NIRS) to predict the protein and energy digestibility of lupin kernel meals when fed to rainbow trout, *Oncorhynchus mykiss*. *Aquaculture Nutrition*, 21: 54–62. doi: 10.1111/anu.12137, which has been published in final form at [http://dx.doi.org/10.1111/anu.12137](http://dx.doi.org/10.1111/anu.12137). This article may be used for non-commercial purposes in accordance With Wiley Terms and Conditions for self-archiving.
Using Near Infrared Reflectance Spectroscopy (NIRS) to predict the protein and energy digestibility of lupin kernel meals when fed to rainbow trout, *Oncorhynchus mykiss*.

Brett Glencross\(^1,5\)*, Nicholas Bourne\(^4\), Wayne Hawkins\(^3,5\), Max Karopoulos\(^3,5\), David Evans\(^2,5\), Neil Rutherford\(^2,5\), Peter McCafferty\(^4,5\), Ken Dods\(^4,5\), Peter Burridge\(^3,5\), Chris Veitch\(^3,5\), Sofia Sipsas\(^3,5\), Bevan Buirchell\(^3,5\), Mark Sweetingham\(^3,5\)

1. CSIRO Food Futures Flagship and Division of Marine and Atmospheric Research, PO Box 120, Cleveland, QLD 4163, Australia
2. Department of Fisheries – Research Division, PO Box 20, North Beach, WA 6020, Australia
3. Department of Agriculture – Government of Western Australia, Baron Hay Court, South Perth, WA 6150, Australia
4. Chemistry Centre of Western Australia, 125 Hay St, East Perth, WA 6001, Australia
5. Aquaculture Feed Grains Program, Centre for Legumes in Mediterranean Agriculture (CLIMA), University of Western Australia, Crawley, WA 6909, Australia

Submitted to: Aquaculture Nutrition

Running Title: Using NIRS to predict feed grain digestibilities
Abstract
This study examined the potential of using near infrared spectroscopy (NIRS) to predict nutrient composition, energy value and digestibility parameters (digestible protein and digestible energy) of lupin kernel meals when fed to rainbow trout. A series of 136 lupin kernel meals were assessed for their protein and energy digestibilities using the diet-substitution approach in a series of 10 experiments over a six-year period from 2002 to 2008. Two reference diets were also included in each experiment. Minimal variance in the digestibility parameters of both reference diets was observed among the experiments ensuring that there was a high degree of robustness in the across-experiment evaluations. The same lupin kernel meal samples were also scanned using a diode array near infrared spectrophotometer (DA-NIRS). The spectra were obtained by the DA-NIRS and were chemometrically calibrated against both the chemical composition and the digestible value data using multivariate analysis software. The results in terms of standard error of cross validation (SECV) and correlation coefficient (R²) show good relationships (R² > 0.8) between the predicted and observed parameters for most of the chemical and digestible value parameters assessed. This study therefore demonstrates that within one raw material type that not only does significant variability in the digestible value of the raw materials exist, but that it is possible to use NIRS technology to provide rapid estimates of the digestible value of those raw materials in near real-time.
Introduction

Lupin meals are one of many raw materials that have consistently been shown to provide sound nutritional value in the diets of a range of aquaculture species (De la Higuera et al., 1988; Burel et al., 1998; Glencross et al., 2005; 2011b; Smith et al., 2008). This raw material has also shown interesting functional properties in extruded feeds in addition to its nutritional attributes adding extra value above their inherent nutritional values (Glencross et al., 2011a; b).

However, like all raw materials, the composition of lupin meals can vary considerably depending on a range of genetic, environmental and processing factors (and of course the interaction between these) (Longnecker et al., 1998; Glencross et al., 2007a;b; 2008a;b). Importantly, this variability in composition has also been noted to extend to the digestible value of lupin meals (Glencross et al., 2008b). This digestible value of lupin meals and indeed, that of most plant proteins is usually a direct reflection of their digestible protein and/or energy content (Burel et al., 1998; Glencross et al., 2008b, 2011a; b). Accordingly any variability in these digestible value parameters (protein and energy) of these meals should translate to variability in their economic value.

In lupin meals, an increase in protein concentration is typically reciprocated by a decrease in the levels of non-starch polysaccharides (NSP) (Glencross et al., 2007b; 2008b). However, high levels of some types of NSP have been implicated in lower nutritional value of lupin meals (Glencross, 2009). In particular the level of lignin has been implicated as a negative factor in protein digestibility via both multivariate analysis and empirical means (Glencross et al., 2008b; 2012). Furthermore, because lupin meals are largely devoid of starch it is recognised that the nutritional value of these raw materials is largely dependent on their protein and lipid components (Glencross et al., 2007b; 2008b). This lends the application of these raw materials to the development of rapid analysis assessments of nutritional value such as the use of near infrared spectroscopy (NIRS). This is particularly pertinent given that virtually all modern aquaculture diets are formulated on a digestible nutrient and energy basis (Glencross et al., 2011a). Therefore an improved assessment of the nutritional value of these raw materials, and on a near-real-time basis, will provide significant advancements in the responsiveness and cost savings in diet formulation by the aquaculture feed industry.

This study reports on the evaluation of the digestibility of a large number of meals of narrow-leaf lupins, *Lupinus angustifolius* when fed to rainbow trout (*Oncorhynchus mykiss*). Part of this dataset is that used in Glencross et al., (2008b), but it is further expanded by an additional 60 samples to provide the critical mass of data to enable potential NIRS calibrations to be developed. The variability in this data set (digestible nutrient/energy values and chemical composition) was studied using diode array near infrared spectroscopy (DA-NIRS). Based on this DA-NIRS analysis of each lupin meal this study reports on this potential of DA-NIRS to predict nutrient composition, energy value and protein and energy digestibility of narrow-leaf lupin kernel meals.
Materials and Methods

Ingredient and diet development

Over a six-year period (2002 – 2008), separate batches of seed of *Lupinus angustifolius* were collected from the Department of Agriculture’s (Western Australia) germ plasm and breeding lines. This seed in many cases constituted the same genotype over several seasons, often from the same site. Samples of the seed were then split using a small disc-mill and aspirated to separate hulls from kernels. A final manual clean of the kernels to remove any remaining hull material was also undertaken on each sample to ensure 100% purity of each kernel preparation. Each kernel sample was then milled using a RetschTM ZM200 rotor mill (Retsch Pty Ltd, North Ryde, NSW, Australia) with a 750 μm screen to create consistent particle sized kernel flour. Additional meals were created based on whole-seed and blended seed and kernel meals and were also included in the sample set. In addition to the lupin meals, each of the other ingredients used in this study was thoroughly ground such that they each passed through a 750 μm screen.

The experiment design was based on a strategy that allowed for the diet-substitution digestibility method to be used (Glencross et al., 2007a). To achieve this a basal diet was formulated and prepared to include approximately 500 g kg⁻¹ DM protein, 210 g kg⁻¹ DM fat and an inert marker (yttrium oxide at 1 g kg⁻¹) (Table 1). Each test ingredient being studied was added at 30% inclusion to a 70% sub-sample of the basal mash (see Table 1). The diets were then processed by addition of water (about 30% of mash dry weight) to the combined mash during 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 before being allowed to cool to ambient temperature in the oven. The basal diet for each experiment was prepared in a similar manner, but without the addition of any test ingredient. In addition a reference lupin kernel meal was included in every digestibility study to allow for cross-comparison across all studies. The basal diet and an example test diet formulations and their composition are presented in Table 1.

Fish handling and faecal collection

These digestibility studies constituted ten separate experiments. Each experiment had two common diets, which included the reference diet and a reference lupin kernel meal (cv. Myallie 2002 season from Coorow). For each experiment hatchery-reared rainbow trout (*Oncorhynchus mykiss*, Pemberton heat-tolerant strain, Western Australia) were transferred from grow-out ponds to experimental tanks (200 L) before being introduced to the experimental diets. Freshwater (salinity < 1 PSU) of 16.0 ± 0.1°C (mean ± S.D. across each of the ten experiments) at a flow rate of about 4 L min⁻¹ was supplied to each of the tanks. For each experiment the tanks were stocked with 15-20 trout of 254 ± 62.5 g (mean ± S.D.; n = 10 experiments). Treatments were randomly assigned amongst 48 tanks within each experiment, with each treatment having three replicates.
Fish were manually fed the diets once daily to apparent satiety as determined over three separate feeding events between 1500 and 1600h each day. The fish were allowed to acclimatise to the allocated dietary treatment for seven days before faecal collection commenced consistent with earlier studies by this group (Glencross et al., 2005; 2007b; 2008a; b). Faeces were collected using manual stripping techniques based on those reported by Glencross et al. (2005; 2007b). Stripped faeces were collected during 0800 to 1000h over a four-day period, with each fish only being stripped twice and not on consecutive days. Faecal samples collected from different days were pooled within tank, and kept frozen at –20°C before being freeze-dried in preparation for analysis.

**Chemical and digestibility analysis**

All chemical analyses were carried out by official NATA (National Association of Testing Authorities) accredited analytical service providers (Chemistry Centre (WA), East Perth, WA, Australia and Animal Health laboratories, Department of Agriculture and Food Western Australia, South Perth, WA, Australia) to AOAC (2005) standards. In this regard each of the diet and faecal samples were analysed for dry matter, yttrium, ash, phosphorus, nitrogen and gross energy content. The dry matter of each sample was calculated by gravimetric analysis following oven drying at 105°C for 24 h. Total yttrium concentrations were determined after mixed acid digestion using inductively coupled plasma atomic emission spectrophotometry (ICP-AES). Protein levels were determined based on the measurement of total nitrogen content of each sample using a LECO auto-analyser, and based on N x 6.25. The amino acid composition of samples was determined by an acid hydrolysis prior to separation via HPLC. The acid hydrolysis destroyed tryptophan making it unable to be determined using this method. Total lipid content of the diets was determined gravimetrically following extraction of the lipids using the chloroform:methanol (2:1). The gross ash content of each sample was determined gravimetrically following the loss of mass after combustion of a sample in a muffle furnace at 550°C for 12 h. Crude dietary fibre was determined by digesting the defatted sample with multiple washes of acetone and ethanol. The resulting residue was corrected for undigested protein and ash. To determine neutral-detergent fibre (NDF) content samples were then boiled with buffered NDF solution. The residue was collected on a coarse sintered glass crucible. The acid-detergent fibre (ADF) was determined following a sample being reacted in 0.5M acid detergent solution and the residue is collected on a coarse sintered glass crucible. The lignin content was determined by reacting the ADF residue with cold 72% sulphuric acid. The sample was then ashed and the residue measured gravimetrically. Gross energy content of each sample was determined by adiabatic bomb calorimetry. Differences in the ratios of the parameters of dry matter, protein, amino acids or gross energy relative to yttrium content, in the feed and faeces in each sample were calculated to determine the apparent digestibility coefficient ($\text{ADC}_{\text{diet}}$) for each of the nutritional parameters examined in each sample of each diet based on the following formula as reviewed in Glencross et al. (2007a):
where \( Y_{\text{diet}} \) and \( Y_{\text{faeces}} \) 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 (dry matter, protein or energy) content of the diet and faeces respectively. The digestibility values for each of the test ingredients in the test diets examined in this study were calculated according to the formulae:

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

NIRS scanning and chemometrics

A Diode Array Near Infrared Spectrometer (DA7200, Perten Instruments, Huddinge, Sweden) was used to scan each of the 135 lupin meals samples. These samples were scanned in reflectance mode using the rotating 75mm sample cup. The spectra from all of the samples were collected across the full wave length range (950 to 1650nm) of the instrument as absorbance at a resolution of 2nm using 9 scans per sample (DA7200 Operation Manual, 2007). The scans were collected in groups of 3 with the sample cup repacked between each group. There were processed by the DA7200 to provide a single spectra for analysis. These spectra were then combined with the nutrient composition, energy value and digestibility data which was copied in to the UNSCRAMBLER® multivariate analysis software package ready for calibration model development. The raw lupin meal spectra obtained is shown in Figure 1.

Initially all the primary spectra were examined visually to eliminate anomalous scans before being copied into the UNSCRAMBLER® multivariate analysis software (Workman and Weyer,
The reference data was then incorporated to form the calibration data set. Cross validation was then used to evaluate the relationship between the spectra and the digestibility, nutrient and energy values. The UNSCRAMBLER® was then used to develop a model that provided a regression based on the whole spectra after specific mathematical treatments of the data. The calibration was evaluated by the statistical measurements of the standard error of cross validation (SECV) and the correlation coefficient ($R^2$) (Workman & Weyer, 2008). An optimisation program was used to determine the math pre-treatments and wave number ranges to use with the data that gave the lowest standard error of cross validation (Workman & Weyer, 2008). Cross validation tests were run on the suggested combinations after taking into consideration any Wave Number ranges known to be appropriate for the parameter under consideration. Validation tests were then re-run after excluding outliers (samples the software flags as either bad reference results or extremely unusual spectrally) (Esbensen, 2004). This process was continued until a balance was struck that included the following elements; a) the standard error of cross validation (SECV) is similar to the standard error of the reference method, b) the number of outliers (poor prediction samples) remaining is small enough or their residual values are low enough to still be able to meet the objectives of the calibration, and c) the correlation coefficient ($R^2$) is sufficiently close to a perfect correlation of 1.0 to indicate probable future robustness and to meet the objectives of the calibration (Esbensen, 2004). Provided the SECV value is in the order of the reference method standard error values of $R^2$ of 0.6 or even lower can be acceptable but values of over 0.8 are desirable (Workman & Weyer, 2008). Also for calibration robustness the standard deviation of the total population used in the calibration model should be at least 1.5x (preferably 2 times or more) the SECV value (Workman & Weyer, 2008).

Statistical analysis

All values are means unless otherwise specified. Figures were constructed and Multivariate chemometric analysis was undertaken using the UNSCRAMBLER® software.
Results and Discussion

Variability exists in all ingredients. This variability can be managed through a variety of means, either by the ingredient supplier, or by the feed manufacturer. Examples of this include the large-scale blending by commodity handlers of grains of different protein levels to produce a more homogenous product, or the analysis of batch variation by feed manufacturers to allow precise customisation of each diet according to each batch of ingredients supplied (Jiang, 2001; Glencross et al., 2008a). In addition to these ingredient management strategies an improved understanding of the level of variability in the chemical composition of the ingredient and how that variability contributes to changes in nutritional value is a key step to maximising the potential value of the ingredient (Glencross et al., 2008a; b). In this study a series of 136 lupin (L. angustifolius) meal samples were collected over a six-year period and examined in a series of digestibility assays with rainbow trout. This work builds on earlier datasets already published by these authors (Glencross et al., 2008a; b), by adding an additional 60 samples and also further analysing all 136 of the samples using NIRS technology.

Data variance

Over a series of ten independent experiments both the basal and reference diets had minimal variability in their digestibility parameters among experiments (Table 2). Dry matter diet digestibilities were different for both diets, but had a similar coefficient of variance of 1.9% and 2.3%. Coefficients of variance (CV) for diet protein digestibility were low at 1.2% and 1.3%, and the means identical at 0.905. Diet energy digestibilities were different for both diets (0.899 and 0.812), but also had low CV’s of 1.4 and 1.9%. These data are consistent with other similar such data published on lupin meal digestibility in rainbow trout (Glencross et al., 2005; 2007b; 2008b; 2011b).

The variability of the ingredient apparent digestibility coefficients for the reference were typically greater than that observed of the diet digestibilities (Table 2). This is to be expected because of the inherent nature in which the ingredient digestibility values are calculated depending on the diet digestibility values of two separate diets and the ingredient composition values. As such this parameter incorporates the errors of all three of these assessments (as reviewed by Glencross et al., 2007a). However, the resultant data still proved to be very robust and from it energy digestibility was the most consistent of the ingredient parameters evaluated, with a CV of 4.0%. Digestibilities for the dry matter had the highest variability with a CV of 9.8%.

There was substantial variability in the composition of the lupin kernel meals used in this study. As a summary of Table 3; the mean ± S.D., protein (N x 6.25) concentration in lupin meals, across all 136 samples was 445 ± 66 g kg⁻¹ on a dry basis (range 27.7 to 61.3 g kg⁻¹). Total lipid was 79 ± 14 g kg⁻¹ (range 50 to 171 g kg⁻¹) and ash 32 ± 7 g kg⁻¹. Carbohydrates, measured by difference
between dry matter minus protein, lipid and ash, were 426 ± 50 g kg\(^{-1}\) on a dry basis (range 254 to 539 g kg\(^{-1}\)). Mean gross energy was 20.6 ± 0.6 MJ kg\(^{-1}\) DM (range 18.7 to 23.0 MJ kg\(^{-1}\) DM). Dietary crude fibre was 309 ± 4.6 g kg\(^{-1}\) on a dry basis (range 175 to 434 g kg\(^{-1}\)), acid-detergent fibre was 101 ± 52 g kg\(^{-1}\) on a dry basis (range 52 to 262 g kg\(^{-1}\)), neutral-detergent fibre was 64 ± 44 g kg\(^{-1}\) on a dry basis (range 30 to 200 g kg\(^{-1}\)) and lignin was 6.2 ± 3.5 g kg\(^{-1}\) on a dry basis (range 2.2 to 21.7 g kg\(^{-1}\)) (Table 3). These composition values are similar, but a little more variable than that of most lupin studies published (De la Higuera et al., 1988; Burel et al., 1998; Glencross et al., 2005; 2007b; 2008b; 2011b).

Substantial variability in ingredient digestible protein/energy parameters was measured across all experimental ingredients (Table 3). This variability was compounded by the combined variability in ingredient composition and ingredient digestibility. The ingredient digestible protein had a coefficient of variation of 15.3%, with a range in digestible protein levels of 244 to 595 g kg\(^{-1}\) on a dry basis (Table 3). The ingredient digestible energy levels had a coefficient of variation of 14.9%, with a range in ingredient digestible energy of 7.7 to 20.5 MJ kg\(^{-1}\) on a dry basis (Table 3). These digestible nutrient values are similar, but a little more variable than that of most other lupin studies published (Burel et al., 1998; Glencross et al., 2005; 2007b; 2008b; 2011b).

**NIRS calibration statistics**

Calibrations were successfully developed for each of the parameters in this study (Table 3). Although the focus of this was on calibration development for the digestible protein and digestible energy values (Figures 3 and 4; Table 3), calibrations were also successfully developed for a suite of compositional features (Table 3). Among the composition calibrations the number of factors used to derived the calibration varied from 3 (sum of amino acids) to 9 (energy). The calibration R\(^2\) values ranged from 0.739 for sum of amino acids to 0.991 for neutral detergent fibre. The cross validation R\(^2\) values were closely aligned with the calibration R\(^2\) values, albeit typically a little weaker. The standard error of cross validation ranged from 0.20 for ash to 2.00 for protein.

Among the digestible protein and digestible energy calibrations the number of factors used to derived the calibration varied from 5 (digestible energy) to 8 (digestible protein). The calibration R\(^2\) values ranged from 0.803 for digestible protein to 0.807 for digestible energy. As with the compositional parameters, the cross validation R\(^2\) values were again closely aligned with the calibration R\(^2\) values, albeit typically a little weaker. The standard error of cross validation ranged from 2.73 for digestible energy to 3.32 for digestible protein.

These digestible protein and digestible energy calibrations appear to be quite unique within the scientific literature. Not only are they the only such calibrations found for a feed grain for these digestible value parameters in fish, they also appear to be relatively unique within broader monogastric research in that they base the development of the calibrations for a single type of feed
grain. This is in contrast to similar such work done with pigs which used a range of cereal varieties (barely, wheat, sorghum, triticale and maize) (van Barneveld et al., 1999). These earlier pig studies also had problems with sample analysis by different laboratories and the lack of inter-experimental reference ingredients, issues that have both been addressed in the present study.

It has been suggested that an acceptable calibration should have an accuracy >1.5 times the value reported as the standard error of the reference method used for that parameter, a value referred to as the RPD (Workman & Weyer, 2008). Based on this assessment the digestible energy calibration had a RPD of 1.50 and the digestible protein a RPD of 1.18. Therefore this would suggest that the digestible energy calibration is acceptable, but that the digestible protein calibration still needs further refinement, despite having a R^2 > 0.80. This refinement could potentially be achieved by either fortifying the dataset further with additional samples and digestibility data, or by removing those data points from the calibration that weaken the calibration R^2.

**Conclusions**

The cross validation tests used in this study provide a valid indication of the potential to predict the nutrient composition, energy value and digestible protein or digestible energy values of the lupin kernel meals as used in the rainbow trout feeding trials. Overall the standard errors of cross validation (SECV) of the parameters investigated were generally commensurate with the cross trial variation seen in the reference sample (cv Myallie) and the RPD values at or close to values indicating robust calibrations for the two target parameters of digestible protein and digestible energy.

This study therefore demonstrates that there is great potential to use NIRS to predict both the composition and digestible values of kernel meal samples of narrow-leaf lupins either by scanning the kernel meal before diet preparation.

**Acknowledgements**

We acknowledge the financial support of the Centre for Legumes in Mediterranean Agriculture (CLIMA) and the Grains Research & Development Corporation. The animal work of this study was undertaken at the Department of Fisheries - Western Australia freshwater research station at Pemberton with thanks to Tony Church and Terry Cabassi for supporting the on-growing of the fish in preparation for this work.
References

AOAC (Association of Official Analytical Chemists). (2005) Official Methods of Analysis of the Association of Official Analytical Chemists. 19th Edition. Association of Official Analytical Chemists. Washington, DC, USA.

Burel, C., Boujard, T., Tulli, F. & Kaushik, S. (2000) Digestibility of extruded peas, extruded lupin, and rapeseed meal in rainbow trout (*Oncorhynchus mykiss*) and turbot (*Psetta maxima*). Aquaculture 188, 285-298.

De la Higuera, M., Garcia-Gallegro, M., Sanz, A., Cardenete, G., Suarez, M.D. & Moyano, F.J. (1988) Evaluation of lupin seed meal as an alternative protein source in feeding of rainbow trout (*Salmo gairdneri*). Aquaculture 71: 37-50.

Esbensen, K.H. (2004) Multivariate data analysis – practice. An introduction to multivariate data analysis and experimental design. 5th Edition. Camo Software, Oslo, Norway. pp598.

Glencross, B.D., (2009) The influence of soluble and insoluble lupin non-starch polysaccharides on the digestibility of diets fed to rainbow trout (*Oncorhynchus mykiss*) Aquaculture 294, 256-261. 3.

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

Glencross, B.D., Booth, M. & Allan, G.L., (2007a) A feed is only as good as its ingredients – A review of ingredient evaluation for aquaculture feeds. Aquaculture Nutrition 13, 17 – 34.

Glencross, B.D., Hawkins, W.E., Vietch, C., Dods, K., McCafferty, P. & Hauler, R.C., (2007b) Assessing the effect of dehulling efficiency of lupin (*Lupinus angustifolius*) meals on their digestible nutrient and energy value when fed to rainbow trout (*Oncorhynchus mykiss*). Aquaculture Nutrition 13, 462-470.

Glencross, B.D., Hawkins, W.E., Evans, D., Rutherford, N., McCafferty, P., Dods, K., & Sipsas, S. (2008a) Assessing the implications of variability in the digestible protein and energy value of lupin kernel meals when fed to rainbow trout, *Oncorhynchus mykiss*. Aquaculture 277, 251-262.
Glencross, B.D., Hawkins, W.E., Evans, D., Rutherford, N., McCafferty, P., Dods, K., Karopoulos M., Veitch, C., Sipsas, S. & Buirchell, B. (2008b) Variability in the composition of lupin (Lupinus angustifolius) meals influences their digestible nutrient and energy value when fed to rainbow trout (Oncorhynchus mykiss). Aquaculture 277, 220-230.

Glencross, B.D., Rutherford, N.R. & Hawkins, W.E., (2011a) A comparison of the growth performance of rainbow trout (Oncorhynchus mykiss) when fed soybean, narrow-leaf or yellow lupin kernel meals in extruded diets. Aquaculture Nutrition 17, e317-e325.

Glencross, B.D., Hawkins, W.E., Evans, D., McCafferty, P., Dods, K., & Hauler, R., (2011b) A comparison of the effect of diet extrusion or pelleting on the digestibility of grain protein products when fed to rainbow trout (Oncorhynchus mykiss). Aquaculture 312, 154–161.

Glencross, B.D., Rutherford, N.R. & Bourne, N., (2012). The influence of various starch and non-starch polysaccharides on the digestibility of diets fed to rainbow trout (Oncorhynchus mykiss). Aquaculture 357, 141–146.

Jiang, Z., (2001) Ingredient variation: Its impact and management. In: van der Poel, A.F.B., Vahl, J.L., Kwakkel, R.P. (Eds.) Proceedings of the 1st World Feed Conference, 7-8 November 2001, Utrecht, Netherlands. pp 47 – 56.

Longnecker, N., Brennan, R. & Robson, A., (1998) Lupin nutrition. In: J.S. Gladstones, C.A. Atkins and J. Hamblin (Eds.) Lupins and Crop Plants: Biology, Production, and Utilisation. CABI Publishing, Cambridge, UK. pp 475.

Perten (2007) DA7200 Diode Array Analyzer – Operation Manual. Perten, Huddinge, Sweden. pp87.

Smith, D.M., Tabrett, S.J., Glencross, B.D., Irvin, S.J. & Barclay, MC. (2007) Digestibility of lupin kernel meals in feeds for the black tiger shrimp, Penaeus monodon. Aquaculture 264, 353-362.

van Barneveld, R.J., 2001. Managing the nutritional quality of stockfeed ingredients. In: van der Poel, A.F.B., Vahl, J.L., Kwakkel, R.P. (Eds.) Proceedings of the 1st World Feed Conference, 7-8 November 2001, Utrecht, Netherlands. pp 31 – 46.
van Barneveld, R., Nuttall, J., Flinn, P.C. & Osborne, B. (1998) NIR reflectance measurement of the digestible energy content of cereals for growing pigs. Journal of Near Infrared Spectroscopy 7, 1-7.

Workman, J. & Weyer, L., (2008) Practical guide to interpretive near-infrared spectroscopy. CRC Press, Boca Raton, FL, USA. pp332.
Tables and Figures

Figure 1. Raw spectral data of *L. angustifolius* kernel meals (n=135) in the NIRS range.
Figure 2. 1st-order derivative of spectral data of *L. angustifolius* kernel meals in the NIRS range.
Figure 3. Measured versus NIRS predicted crude protein of *L. angustifolius* kernel meals (blue data). Shown in red is the cross-validation dataset.
Figure 4. Measured versus NIRS predicted digestible protein value of *L. angustifolius* kernel meals (blue data). Shown in red is the cross-validation dataset.
Figure 5. Measured versus NIRS predicted digestible energy value of *L. angustifolius* kernel meals (blue data). Shown in red is the cross-validation dataset.
### Table 1. Formulation of key diets in each experiment

|                     | Basal | Test  | Reference |
|---------------------|-------|-------|-----------|
| Fishmeal            | 700.0 | 490.0 | 490.0     |
| Fish oil            | 150.0 | 105.0 | 105.0     |
| Test meal*          | -     | 300.0 | -         |
| Reference meal#     | -     | -     | 300.0     |
| Wheat flour         | 144.0 | 100.8 | 100.8     |
| Vitamin and mineral premix | 5.0 | 3.5 | 3.5 |
| Yttrium oxide       | 1.0   | 0.7   | 0.7       |
| **Total**           | 1000.0| 1000.0| 1000.0    |

*one of 135 test lupin meals

*L. angustifolius cv. Myallie kernel meal. Coorow Seed Cleaners, Coorow, WA, Australia*
Table 2. Digestibility data variation

|                        | Dry matter | Protein | Energy |
|------------------------|------------|---------|--------|
| Diet digestibility – Basal reference diet |            |         |        |
| Mean                   | 0.822      | 0.905   | 0.899  |
| SD                     | 0.019      | 0.012   | 0.013  |
| SEM                    | 0.007      | 0.004   | 0.005  |
| CV%                    | 2.3%       | 1.3%    | 1.4%   |

| Diet digestibility - *L. angustifolius* cv Myallie reference |            |         |        |
| Mean               | 0.723      | 0.905   | 0.812  |
| SD                 | 0.014      | 0.011   | 0.016  |
| SEM                | 0.004      | 0.003   | 0.005  |
| CV%                | 1.9%       | 1.2%    | 1.9%   |

| Ingredient digestibility - *L. angustifolius* cv Myallie reference |            |         |        |
| Mean               | 0.499      | 0.971   | 0.564  |
| SD                 | 0.049      | 0.039   | 0.041  |
| SEM                | 0.014      | 0.011   | 0.012  |
| CV%                | 9.8%       | 4.0%    | 7.3%   |

| Ingredient digestibility - *L. angustifolius* : All test samples |            |         |        |
| Mean               | 0.546      | 0.933   | 0.614  |
| SD                 | 0.136      | 0.073   | 0.113  |
| SEM                | 0.011      | 0.006   | 0.009  |
| CV%                | 25.1%      | 7.8%    | 18.4%  |

CV%: Coefficient of variation = SD / Mean x100
Table 3. Nutrient composition, digestible value data and NIRS calibration statistics

| Parameters                  | Sample characteristics |                  |                  |                  | Calibration statistics |                  |
|-----------------------------|------------------------|------------------|------------------|-------------------|------------------------|------------------|
|                             |                        | n    | Mean | SD   | CV%  | Min  | Max   | Factors | Cal R^2 | Val R^2 | SECV |
| **Composition**             |                        |      |      |      |      |      |       |         |         |         |      |
| Dry Matter                  | 136                     | 919  | 13   | 1.5% | 892  | 950  |        | 8       | 0.933   | 0.908   | 2.00  |
| Protein (N * 6.25)          | 136                     | 445  | 66   | 14.9%| 277  | 613  |        | 5       | 0.868   | 0.782   | 0.48  |
| Total Lipid                 | 109                     | 79   | 14   | 17.6%| 50   | 171  |        | 5       | 0.884   | 0.784   | 0.20  |
| Ash                         | 110                     | 32   | 7    | 21.8%| 19   | 66   |        | 5       | 0.868   | 0.772   | 1.70  |
| Carbohydrates               | 109                     | 426  | 50   | 11.8%| 254  | 539  |        | 4       | 0.868   | 0.772   | 1.70  |
| Energy                      | 136                     | 206  | 6    | 3.0% | 187  | 230  |        | 9       | 0.885   | 0.844   | 1.70  |
| Sum of Amino Acids          | 93                      | 443  | 37   | 8.3% | 332  | 570  |        | 3       | 0.739   | 0.686   | 1.74  |
| Crude Fibre                | 35                      | 309  | 46   | 14.9%| 175  | 434  |        | 4       | 0.980   | 0.942   | 0.96  |
| Acid Detergent Fibre       | 38                      | 101  | 52   | 51.1%| 52   | 262  |        | 5       | 0.983   | 0.968   | 0.88  |
| Neutral Detergent Fibre    | 38                      | 64   | 44   | 68.5%| 30   | 200  |        | 5       | 0.991   | 0.984   | 0.74  |
| Lignin                     | 38                      | 6.3  | 3.5  | 56.1%| 2    | 22   |        | 5       | 0.932   | 0.920   | 0.42  |
| **Digestible Value**        |                        |      |      |      |      |      |       |         |         |         |      |
| Digestible Protein         | 136                     | 414  | 63   | 15.3%| 244  | 595  |        | 8       | 0.803   | 0.735   | 3.32  |
| Digestible Energy          | 136                     | 122  | 18   | 14.9%| 77   | 205  |        | 5       | 0.807   | 0.778   | 2.73  |