

# USING NEAR-INFRARED REFLECTANCE SPECTROSCOPY (NIRS) TO PREDICT THE DIGESTIBLE PROTEIN AND ENERGY VALUE OF LUPIN KERNEL MEALS WHEN FED TO RAINBOW TROUT, *ONCORHYNCHUS MYKISS*

Brett Glencross<sup>1,4</sup>, Wayne Hawkins<sup>2,4</sup>, Peter Burridge<sup>2,4</sup>, David Evans<sup>1,4</sup>, Neil Rutherford<sup>1,4</sup>,  
Peter McCafferty<sup>3,4</sup>, Ken Dods<sup>3,4</sup> and Sofia Sipsas<sup>2,4</sup>

<sup>1</sup>Department of Fisheries, PO Box 20, North Beach WA 6920, Australia

<sup>2</sup>Department of Agriculture and Food Western Australia, Baron-Hay Court, South Perth WA 6150, Australia

<sup>3</sup>Chemistry Centre of Western Australia, 125 Hay St, East Perth WA 6001, Australia

<sup>4</sup>Centre for Legumes in Mediterranean Agriculture, University of Western Australia, Crawley WA 6909, Australia

Corresponding author's email: [Brett.Glencross@fish.wa.gov.au](mailto:Brett.Glencross@fish.wa.gov.au)

## ABSTRACT

As with all raw materials, learning to manage variability is one of the major limitations to improved adoption and maximisation of the value of lupins. Over a five-year period, 10 separate digestibility experiments were undertaken to examine the digestibility of protein and energy from 136 different samples of lupin meal. The digestibility of protein and energy to rainbow trout (*Oncorhynchus mykiss*) was assessed for each of the meals. Digestible protein values ranged from 304 to 547 g/kg DM and digestible energy values ranged from 9.9 to 14.5 MJ/kg DM. The digestible protein and energy values were then assessed using multiple regression techniques to determine which compositional parameters accounted for the majority of the variability. This study demonstrated that within one raw material type not only does significant variability in the digestible value of lupin meals exist, but that it is possible to identify compositional features of that raw material that are intrinsically influencing their digestible value. Further assessment of these samples using near-infrared spectroscopy (NIRS) shows that there are certain wavelengths that correlate with compositional attributes of the lupin meals relevant to their digestible value. From this work it has been possible to develop a calibration to predict both digestible protein and energy content of lupin kernel meals.

## KEYWORDS

quality, variability, NIRS, aquaculture, fish, digestibility

## INTRODUCTION

In order to reduce risk associated with being too dependent on fish meal and oil use in aquaculture feeds, a range of grain protein meals has been assessed and developed as alternatives. From these initiatives lupins have emerged as one the main grain sources now being

used in aquaculture feeds throughout the world. However as with all raw materials, learning to manage variability is one of the major limitations to improved adoption and maximisation of the value of these raw materials (Glencross *et al.* 2007a).

The nutritional value of lupin grain, and indeed that of most plant proteins, is usually a direct reflection of their digestible protein and/or energy content (Glencross *et al.* 2004) Accordingly any variability in the digestible value of the meals can translate to variability in their economic value (Glencross *et al.* 2008b). In lupins, an increase in protein content is usually offset by a concomitant decrease in the levels of non-starch polysaccharides (NSP) (Glencross *et al.* 2008a). High levels of NSP and other fibre types have been implicated in reduced nutritional value of plant protein meals (Glencross *et al.* 2008a). Furthermore because lupins are largely devoid of starch it is hypothesised that only the protein and lipid components of the raw material are contributing to its nutritional value (Glencross *et al.* 2007b).

The key to managing nutritional variability lies in gaining as robust-as-possible data on the chemical composition of the raw material prior to its use in a feed. Near-infrared spectroscopy (NIRS) has been used extensively to measure the crude compositional characteristics of feed grains prior to their use for some time. Modern NIRS equipment can make a range of compositional evaluations in a manner of seconds. Some progress has also been made towards measuring digestible nutrient and energy parameters from some grain varieties for pigs (van Barneveld *et al.* 1998). NIRS provides the opportunity to rapidly and non-destructively predict crude and digestible composition values prior to diet preparation. This study reports on an assessment of NIRS to predict nutrient and energy composition, and digestibility values of narrow-leaved lupin (*Lupinus angustifolius*) kernel meals when fed to rainbow trout.

## MATERIALS AND METHODS

Over a five-year period, 10 separate digestibility experiments with rainbow trout (*Oncorhynchus mykiss*) were undertaken to examine the digestibility of protein and energy from 120 different samples of lupin meal. The initial data produced from the first 75 of these samples was used to evaluate key compositional features affecting digestible value of lupin meals (Glencross *et al.* 2008a). From these initial evaluations the use of NIRS was applied to the samples to evaluate the potential of this technology to predict digestible protein and energy values. The additional 60 samples were used to fortify the NIRS calibration.

### SAMPLE PREPARATION

Lupin samples were obtained from the Australian National Lupin Breeding Program's germplasm lines and selected on the basis of maximal crude protein variability as assessed by existing crude protein NIRS calibrations. 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 the kernel preparation. Each kernel sample was then milled using a Retsch rotor mill with a 750  $\mu\text{m}$  screen to create a kernel flour. In addition to the lupin kernel flours, each of the test ingredients used in this study was thoroughly ground such that they passed through a 750  $\mu\text{m}$  hammer mill screen. Typical particle size was less than 200  $\mu\text{m}$ . Chemically measured crude protein values varied from 365 to 567 g/kg DM. Other compositional parameters assessed included amino acids, total lipids, gross energy, ash, total carbohydrates, cellulose, hemicellulose and lignin.

### DIGESTIBILITY EVALUATION

Each of the 12 experiments had two common diets, which included the reference diet and a reference lupin kernel meal (cv. Myallie). For each experiment hatchery-reared rainbow trout (*Oncorhynchus mykiss*) were transferred from grow-out ponds to experimental tanks (200 L) between three and ten days prior to being introduced to the experimental diets. Freshwater (salinity < 1 PSU) of  $16.0 \pm 0.1^\circ\text{C}$  (mean  $\pm$  S.D.) at a flow rate of about 4 L/min was supplied to each of the tanks. For each experiment the tanks were stocked with 15-20 trout of ~250 g. The trout were allowed to acclimatise to the allocated dietary treatment for seven days before faecal collection commenced using stripping techniques (Glencross *et al.* 2005). The collected samples were stored at  $-20^\circ\text{C}$  before being freeze-dried in preparation for analysis. Triplicate samples were collected for each diet with the mean value used in this study.

### CROSS EXPERIMENT STANDARDISATION

To assess the variation in the ingredient and digestibility data twelve replicates of a reference sample of *Lupinus angustifolius* (cv. Myallie) were included in the study. This provided information about the error background involved in the sampling and analytical techniques. The variation in nutrient and energy digestibility data for both reference diets and the reference sample are given in Table 2. This data is essential for establishing the NIRS calibrations since the SECVs generated can only be validated by comparison to the background errors of the experiment.

### NIR SPECTROSCOPY ASSESSMENT

A Bruker Fourier Transform MPA and the OPUS<sup>®</sup> software package (Ver 5.5, © 2004 Bruker Optik GmbH, Rudolf-Plank-Straße 27, D-76275, Ettlingen) was used to scan 74 kernel flour (and 44 seed) samples in duplicate (Fig. 2). These samples were scanned in a temperature controlled atmosphere with the instrument operated in reflectance mode using the rotating 97 mm sample cup. The spectra from the samples were collected across the full Wave Number range (12,493 to 3,599  $\text{cm}^{-1}$ ) of the instrument as absorbance with a bandwidth of 8  $\text{cm}^{-1}$  using 64 scans per sample. The full set of kernel meal spectra in the Wave Number range used by many of the calibrations is shown in Fig. 2. As part of the evaluation of the NIRS calibrations the inter-correlation of prediction values needed to be examined to ensure that the regressions used were independently derived. In practice some inter-correlation was inevitable since the same spectra were being used and there were inherent relationships in the reference data. (A detailed analysis of the influence of ingredient composition on the digestibility is provided in the paper cited above.)

### CHEMOMETRIC ANALYSIS

The individual spectra were examined visually to eliminate the possibility of any anomalous scans before they were incorporated into the OPUS<sup>®</sup> QUANT multi-variate calibration software (©Bruker Optik, as above). The reference data was then copied into OPUS<sup>®</sup> to form the calibration data set. The spectra were evaluated as the mean of two scans. The OPUS<sup>®</sup> optimisation program, incorporating a partial least square (PLS) fit method, was then used to develop calibration models. This produced regression equations based on selected parts of the spectra after specific mathematical treatments of the data. Cross validation tests were then run for each parameter in turn using the suggested calibration models that incorporated appropriate wave number ranges and math pre-treatments. The calibrations were evaluated by examining the statistical measurements of the standard error of cross validation (SECV) and the correlation coefficient ( $R^2$ ).

An NIRS calibration was only considered viable if the SECV value was similar to the standard error of the reference method and no more than a half (preferably a third or less) of the standard deviation of the data set used to produce the calibration (or future prediction population).  $R^2$  values of 0.6 or even lower can be acceptable in a NIRS calibration, although values of over 0.8 are desirable for calibration accuracy (Bertrand, 2001).

## RESULTS

Over the first series of seven independent experiments both the basal reference and ingredient 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 2.2%. Coefficients of Variance (CV) for diet protein digestibility were low at 0.9% and 1.3%, but the means were similar. Diet energy digestibilities had a similar CV of 1.4% and 1.7% but differed in their mean values. Diet digestibilities of the sum of amino acids were also similar for both diets but had a similar CV of 0.9%, the lowest of the parameters evaluated. Variability of the ingredient apparent digestibility coefficients for the reference ingredient were greater than that observed of the diet digestibilities (Table 2). Energy digestibility was the most consistent of the ingredient parameters evaluated, with a CV of 4.2%. Ingredient digestibilities for the Sum of Amino acids had the highest variability with a CV of 20.6% (Glencross *et al.* 2008a).

**Table 1.** Variability in lupin meal composition (% dry matter) across all test ingredients (n = 75).

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

CV%: Coefficient of variation = SD / Mean x100.

There was substantial variability of the crude composition of the lupin kernel meals used in this study (Table 1). Substantial variability in ingredient digestible nutrient parameters was also measured across all experimental ingredients (Table 3). The nutritional implications of this variability were compounded by the variability in ingredient composition and ingredient digestibility. The key digestibility parameter of ingredient digestible nitrogen had a coefficient of variation of 11.3%, with a range in digestible nitrogen levels of 30.4 to 54.7 (Table 3). The ingredient digestible energy levels had a coefficient of variation of 8.2%, with a range in ingredient digestible energy of 9.9 MJ/kg to 14.5 MJ/kg.

Lupin kernel meal digestible protein (nitrogen digestibility x meal protein content) was significantly affected by the ingredient protein density ( $R = 0.4109$ ,  $P = 0.014$ ) and by reciprocation the ingredient carbohydrate density ( $R = -0.4921$ ,  $P = 0.003$ ). The relationship between protein content and energy also meant that energy density was significantly correlated to digestible protein value ( $R = 0.4836$ ,  $p = -0.003$ ). There was no significant effect of ingredient crude fibre, acid-detergent fibre or neutral-detergent fibre on ingredient protein digestibilities. However, lignin content of the lupin kernel meals had a significant ( $R = -0.4981$ ,  $p = 0.002$ ) effect on the level of digestible protein in the kernel meals (Fig. 1, reproduced from Glencross *et al.* 2008a).

**Table 2.** Mean values and data variance associated with apparent digestibility coefficients of the basal reference diet and the reference ingredient digestibility assessments across all experiments (n = 7).

	Dry matter	Protein	Energy	Sum Amino Acids
<b>Diet digestibility – Basal reference diet</b>				
Mean	0.822	0.905	0.899	0.935
SD	0.019	0.012	0.013	0.008
SEM	0.007	0.004	0.005	0.003
CV%	2.3%	1.3%	1.4%	0.9%
<b>Diet digestibility – <i>L. angustifolius</i> cv. Myallie reference ingredient</b>				
Mean	0.726	0.904	0.804	0.929
SD	0.016	0.008	0.013	0.008
SEM	0.006	0.003	0.005	0.003
CV%	2.2%	0.9%	1.7%	0.9%
<b>Ingredient digestibility – <i>L. angustifolius</i> cv. Myallie reference ingredient</b>				
Mean	0.503	0.982	0.557	0.914
SD	0.039	0.072	0.023	0.188
SEM	0.015	0.027	0.009	0.071
CV%	7.7%	7.4%	4.2%	20.6%

**Table 3.** Variability in ingredient digestibility parameters and digestible values across all test ingredients.

	Mean	SD	CV%	Min.	Max.
<i>Ingredient digestibility coefficients</i>					
Dry matter	0.532	0.050	9.5%	0.391	0.655
N	0.933	0.096	10.3%	0.655	1.146
Fat	0.735	0.593	80.7%	-3.151	1.818
P	1.834	0.884	48.2%	0.126	3.970
Energy	0.573	0.046	8.0%	0.482	0.694
Sum of Amino Acids	0.880	0.130	14.8%	0.526	1.265
<i>Digestible value (% dry basis)</i>					
Dry matter	48.7	4.7	9.6%	35.8	59.8
Protein (N x 6.25)	42.3	4.8	11.3%	30.4	54.7
Fat	5.9	3.3	55.1%	0.0	9.7
P	0.7	0.3	44.0%	0.1	0.6
Energy (MJ/kg dry basis)	11.9	1.0	8.2%	9.9	14.5
Sum of Amino Acids	38.7	6.2	16.1%	23.4	50.6

**Table 4.** NIRS calibration statistics for ingredient digestible values. DPV is digestible protein value and DEV digestible energy value.

	DPV	DEV
Reference Mean (%)	41.3	11.5
Reference SD (%)	3.64	0.52
Population Range (%)	20.4	4.4
Population SD (%)	4.3	1.0
SECV	2.7	0.75
R <sup>2</sup>	0.472	0.355

SD: Standard deviation.

SECV: Standard error of cross validation.

Lupin kernel meal digestible energy values were also significantly affected by a wide variety of ingredient composition parameters. The ingredient protein (nitrogen) density ( $R = 0.4978$ ,  $P = 0.002$ ) significantly influenced the digestible energy content of the ingredient. The ingredient protein + fat density ( $R = 0.5368$ ,  $P = 0.001$ ) had a stronger significantly positive influence on the digestible energy density of the ingredient than the protein content alone. Reciprocating this, the ingredient carbohydrate density ( $R = -0.5421$ ,  $P = 0.001$ ) had a significant negative effect on digestible energy levels. The ingredient energy density had a significant effect ( $R = 0.4164$ ,  $P = 0.013$ ) on the digestible energy level of the ingredient. There was no significant effect of ingredient crude fibre, acid-detergent fibre, neutral-detergent fibre density or lignin content on the ingredient energy digestibilities (Glencross *et al.* 2008a).

Based on the initial 75 samples the development of NIRS calibrations for nutrient and energy digestible value calibrations produced SECVs commensurate with the standard errors seen in the reference data (see Table 2). However for the Digestible Energy calibrations the SECV was considered too high to be valid. The best calibration was that for Digestible Protein, which had a SECV of 2.7% with a  $R^2 = 0.472$ . The standard deviation of the trial population for digestible protein was 4.3%, or just less than twice the SECV. For this calibration the math pre-treatment was Straight Line Subtraction with a Wave Number range of 1249.3 to 9295.7  $\text{cm}^{-1}$  with 2 of the 75 samples removed as outliers.

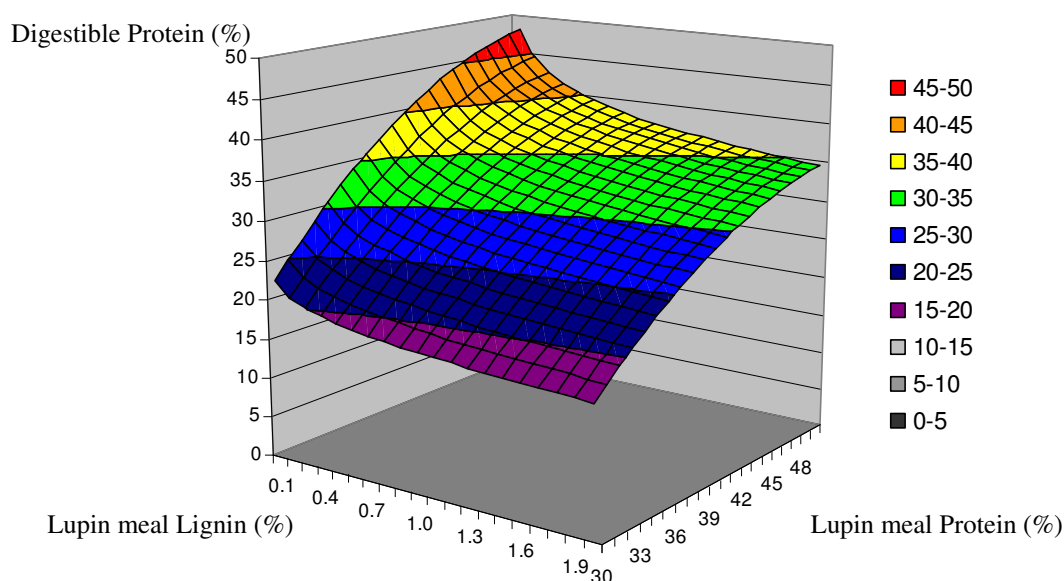
## DISCUSSION

This study demonstrates that within one raw material type not only does significant variability in the digestible value of the raw materials exist, but that it is possible to identify compositional features of that raw material that are intrinsically influencing its own digestible value. Predictive equations have been developed that explain this variability (Glencross *et al.* 2008a). The implications of such variability on the performance of diets fed to fish have also been demonstrated (Glencross *et al.* 2008b).

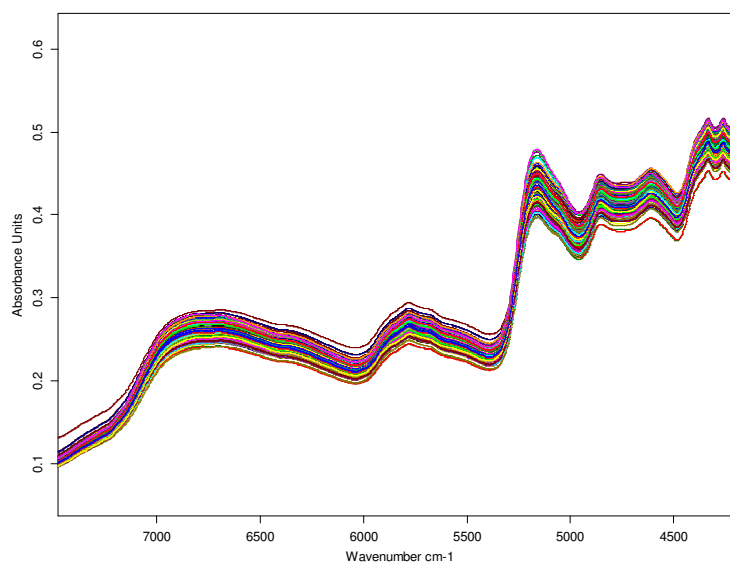
The digestible nutrient and energy content of the lupin meals reflected the combined effects of both ingredient digestibilities and ingredient composition. The digestible protein content of the lupin meals was positively affected by both the protein and energy content, but was negatively affected by lignin and carbohydrate content of the meals. The digestible energy content of the kernel meals was also positively affected by protein and its own energy density, but only negatively affected by carbohydrate content. Multiple regression modelling indicated that, together the ingredient protein and lignin content were the strongest predictors of digestible protein value, explaining close to 60% of the variability in this parameter (Fig. 1). This study demonstrated that within lupin meals that not only does significant variability in the digestible value exist, but that it is possible to identify certain compositional features of that raw material that are intrinsically influencing its own digestible value. These observations are consistent with other studies on pigs that have also reported that the presence of lignin affects digestibility parameters (King and Taverner, 1975).

There have been few studies reported in the literature on the development of calibrations for digestible value parameters of raw materials for aquaculture feeds (Glencross *et al.* 2007a). Some progress in this area has been made for terrestrial species, such as pigs, for determining the digestible energy value of cereals (van Barneveld *et al.* 1998). As to be expected, the digestibility calibration statistics (Table 4.) were not as good as those obtained from the original composition data as they combined the error of both the composition and digestibility assessments. However, the calibration for Digestible Protein Value (DPV) had a SECV at about the level of error in the reference results. However, the Digestible Energy Value (DEV) had a SECV at about the level of error in the successful calibration in terms of this variation. Relatively poor  $R^2$  values were evident for ingredient digestible value parameters compared to the original composition and energy data indicating that the NIRS found it difficult to distinguish the values against the background variation. Thus the DPV calibration appears just short of being viable based on 75 samples, but both the DPV and DEV value calibrations have reasonably low SECVs and would be significantly improved with further fortification of the sample set on which the calibrations are based.

With improvements in the efficiency of aquaculture production systems and the definition of the nutritional requirements for these species there will be an increased need to improve our capacity to manage the nutritional quality of aquaculture feed ingredients. The further development of rapid, accurate and cost-effective analysis of raw materials using NIRS remains one of the most promising options to address this issue.



**Fig. 1.** Model of the dual influence of lupin meal protein (%DM) and lignin (%DM) on the digestible protein content (%DM) of lupin meal when fed to rainbow trout. Derived from Glencross *et al.* (2008a).



**Fig. 2.** Variation in the NIR spectra associated with the different lupin samples used in this study.

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