Final Report

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Canola Meal Value Chain Quality Improvement

Stage 2

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Executive Summary

This project has provided support for the use of NIR technology in predicting reactive lysine as a measure of the quality of canola meal for pig feeding.

A total of 102 samples from commercially available canola meal were analysed for total and reactive lysine. Based upon previous research work, the objective was to develop an NIR calibration that could be used to asses canola meal quality and provide industry with a rapid analysis tool to determine the effects of heat processing upon meal quality.

The results of this project have confirmed that the measure of reactive lysine relative to total lysine provides a measure of meal quality. The NIR calibrations developed are sufficient to differentiate between high and low quality meals. Further work is being undertaken to further improve the calibrations prior to commercial release of the technology.

Additional data relating to protein, moisture, fat and amino acid content of Australian canola meals are provided from this project.

Contents

1 Background

Canola meal is a major protein source for animal feeding in Australia and is used as a feed ingredient by the pig industry across all Australian states. Variation in canola meal protein is a factor in limiting the value of canola meal. The variation is illustrated in Quality of Australian Canola (Seberry et al 2008) in which total crude protein, based upon canola seedstock, can vary from 36 to 47%.

Protein in itself is not a good indicator of canola meal quality, but protein digestibility is a more significant parameter. Heat treatment used by processors to extract oil from canola seed, and subsequent desolventising and toasting is recognised as a major contributor to the loss in protein digestibility (van Barneveld 1998, Newkirk and Classen 2002).

A stage 1 project (Spragg and Mailer 2007) was completed, looking at the variation in nutritional quality of canola meal produced within Australian oilseed crushing plants. Reactive lysine was used as an analysis tool to identify differences in meal quality resulting from different processing parameters used within commercial crushing plants. It was recommended that further work be completed using NIR technology to develop calibrations for reactive lysine and lysine loss due to heat damage during processing.

2 Project Objectives

This project addresses two Pork CRC outputs in providing a more reliable and consistent protein supply for the pig industry.

The project had the following specific objective:

- Establishment of NIR calibrations for canola meal for application by industry to allow rapid assessment of meal quality in terms of heat damage occurring during processing.

Work completed by van Barneveld (1998) demonstrated the potential use of NIR technology to predict protein quality based upon reactive lysine analysis. This work recommended that oilseed processors should consider the adoption of the reactive lysine assay as a quality assurance tool.

NIR calibrations developed within this project are intended for use by:

- Commercial oilseed crushing plants in providing nutritional data to allow oilseed crushing parameters to be adjusted to modify meal quality.
- Feed manufacturers and the pig industry to better define canola meal being utilised within feed formulations.

The ultimate result will be the supply of higher quality and more consistent canola meal for use by the Australian pig industry.

This second stage project is aimed at providing industry with practical tools to better define canola meal quality and for use in potentially modifying processing conditions to improve meal nutritional quality.

3 Methodology

3.1 Sample Collection

Oilseed crushing plants co-operating within the project were required to take canola meal samples which were sent to NSW DPI Wagga Wagga Research Institute.

Samples were collected from six commercial crushing plants, these being:

Crushing Plant	No. samples collected
Cargill Australia - Newcastle NSW	**40**
Cargill Australia - Melbourne Vic	**46**
Riverland Oilseed Processors - Numurkah Vic	**41**
Riverland Oilseed Processors - Pinjarra WA	**39**
Riverland Oilseed Processors - Millicent SA	**40**
MSM Milling - Manildra NSW	**18**
TOTAL samples	**224**

Each crushing plant was required to collect samples over a 3 month time period, the intention being to obtain a wide range of meal samples.

3.2 Initial Sample Screening

All 224 canola meal samples were analysed through an NIR spectrometer (FOSS 6500) generating spectral data. Using NIR sample select ISI software, 102 samples were selected that uniformly covered the variability in the original dataset. Selected were 55 solvent and 47 expeller meal samples.

3.3 Sample Analyses

Samples were analysed for moisture, protein and oil content at NSW DPI Wagga Wagga Research Institute. Total and reactive lysine assays were completed by Massey University, New Zealand.

Moisture
A modified American Oil Chemist Society (AOCS) method Ai-2-75 (AOCS 1998) *Moisture and volatile matter, by air oven in sunflower seed* was used to determine moisture. 10 grams of sample was placed in a 130^0C fan forced oven for 2 hours. Results are reported % moisture as received.

Protein
Samples were analysed as received by Dumas combustion using a Leco analyser using AOCS official method Ba4e-93 (AOCS 1998) *Combustion method for determination of crude protein in animal feeds, oilseed meals and oilseeds*. Results are reported as % protein (nitrogen x 6.25) as received.

Oil
Oil content was determined using the AOCS official method Am 2-93 (AOCS 1998) *Determination of oil content in oilseeds*. Samples were weighed into a cellulose extraction thimble then extracted with hexane on a goldfische apparatus for 4 hours using a previously weighed flask. The thimbles were then dried at room temperature and the sample reground for 10 secs using a coffee grinder. The entire sample is returned to the thimble and extracted again for 2 hrs using the same flask as the previous extraction. The thimble was again dried

and sample reground for 10 sec using a coffee grinder with the entire sample being transferred back to the thimble. The sample was extracted for 2hrs using the previous flask. The solvent was then evaporated and the flask weighed. Results were expressed as % oil as received.

Total and Reactive Lysine

The 102 selected canola meal samples were analysed by Massey University, New Zealand for total and reactive lysine.

Total lysine was determined using a Waters ion exchange HPLC system.

Reactive lysine contents were determined in single 5 mg samples after incubation for 2 days in 0.6 M O-methylisourea, pH 10.6 at 21 ^{o}C in a shaking water bath, with the reagent to lysine ratio being greater than 1000, according to the procedure of Moughan and Rutherfurd (1996). After incubation, the samples were dried using a Speedvac concentrator (Savant Instruments, Inc, Farmingdale, NY, USA) and analyzed for homoarginine using a Waters ion exchange HPLC system, utilizing post-column ninhydrin derivatization and detection using absorbance at 570 nm, following hydrolysis in 6 M glass-distilled HCl containing 0.1% phenol for 24 h at 110$\pm$ 2^{o}C in evacuated sealed tubes. The weight of reactive lysine was calculated from the determined molar quantity of homoarginine and the molecular weight for free lysine.

Reverted lysine represents the difference between total lysine and reactive lysine.

Amino Acid Profile

The selected 102 canola meal samples were analysed for amino acid content from an NIR prediction (Degussa) using an NIR spectrometer (FOSS 5500).

3.4 Calibration Development

NIR calibrations were developed following the instrument manufacturers directions using a Foss 6500 NIR spectrophotometer. Calibrations for nitrogen, protein and moisture were developed using samples from NSW DPI trial samples analysed by wet chemistry methods detailed above.

Initial Lysine Calibration: Calibrations for reactive lysine were developed initially from 60 canola meal samples that were sent to Massey University in New Zealand in 2001 for total and reactive lysine analysis. SARDI used these results to develop NIR calibrations for both analyses. The samples are 40 cold press meal samples that had been artificially treated with an autoclave and dry heat at various temperatures for various times and 20 solvent extracted samples with no treatment.

The samples and the NIR calibration and equation files were sent to NSW DPI Wagga Wagga Research Institute. These samples were scanned into the 6500 NIR at Wagga and a calibration was developed using the same parameters as the original SARDI calibration. The statistics produced were different to the original calibration, suggesting that the samples have changed over time.

To confirm the samples had changed, some samples were sent to Massey University for reactive lysine analysis. The differences were large and the samples from the initial trials were considered unusable.

Second calibration: Finished meal samples (21 samples in total- 9 solvent, 9 expeller and 3 cold press) were sent to Massey University in NZ for reactive lysine analysis. These samples were from the Stage 1 project completed in 2007.

A calibration was developed using all 21 samples (average of duplicate scans) however the calibration statistics indicated that there would be large errors associated with this calibration. This may be due to the large differences in oil content between extraction methods which lead to very different spectra.

Preliminary calibrations were developed for solvent and expeller meals samples separately. Each sample was scanned in duplicate and treated as a separate sample because 9 samples are not sufficient for a calibration. Cold press samples were not used in either calibration.

Third Calibration: Selected 102 canola meal samples (55 solvent and 47 expeller) were sent to Massey University in NZ for reactive lysine analysis. A calibration was developed for reactive lysine and Reactive/Total lysine.

4 Results

4.1 Nutritional Quality

Moisture, protein and oil results shown in Table 1 are in close agreement with those found in Stage 1. The increased efficiency of solvent oil extraction is shown with expeller meal containing on average 5.8% more oil than solvent extracted meal. Expeller meal is 1.4% lower in protein than solvent meal, this difference in Stage 1 was 1.0%.

Table 1. *Chemical Composition of canola meal (as received)*

		Expeller Extracted Meal					Solvent Extracted Meal				
Nutrient	Units	n	Mean	Min.	Max.	SD	n	Mean	Min.	Max.	SD
Moisture	%	94	9.7	6.0	11.6	1.26	116	11.2	8.4	13.4	1.16
Protein	%	91	36.0	32.7	38.2	1.35	116	37.4	34.5	41.0	1.82
Oil	%	94	10.6	8.5	15.8	1.56	116	4.8	2.9	6.6	1.09

Amino acid content of both expeller and solvent canola meal obtained from NIR analysis is provided in Tables 2 and 3. The larger number of samples analysed for amino acids conducted in this Stage 2 project provide results which are in close agreement with those found in Stage 1.

Table 2. *Amino acid content of expeller and solvent extracted canola meal (g/kg, as received)*

	Expeller Extracted Meal n=47				Solvent Extracted Meal n=55			
Amino Acid	Mean	Min.	Max.	SD	Mean	Min.	Max.	SD
Methionine	7.0	6.5	8.1	0.28	7.2	6.7	7.8	0.25
Cystine	8.5	7.6	9.0	0.29	8.6	7.9	9.5	0.32
M+C	15.6	14.8	16.3	0.37	15.9	14.8	17.3	0.52
Lysine	19.6	18.3	20.3	0.47	20.0	17.2	21.0	0.67
Threonine	15.0	14.2	16.5	0.47	15.7	14.8	16.9	0.49
Tryptophan	4.8	4.4	5.6	0.22	5.0	4.5	5.4	0.16
Arginine	21.0	19.6	24.7	0.98	21.8	15.6	24.0	0.80
Isoleucine	13.9	13.0	15.2	0.44	14.3	13.4	15.6	0.51
Leucine	24.3	22.8	26.3	0.76	25.4	23.5	27.8	0.90
Valine	17.9	16.9	19.1	0.50	18.6	17.5	20.1	0.57
Histidine	9.5	8.9	10.0	0.30	9.8	9.0	10.8	0.34
Phenylalanine	13.9	12.8	16.6	0.67	14.6	13.4	15.9	0.49

Table 3. *Amino acid content of expeller and solvent extracted canola meal (% in crude protein)*

	Expeller Extracted Meal n=47	Solvent Extracted Meal n=55	All Meal n=102
Amino Acid	Mean	Mean	Mean
Methionine	2.00	1.96	1.98
Cystine	2.44	2.35	2.39
M+C	4.46	4.34	4.40
Lysine	5.60	5.47	5.53

Threonine	4.29	4.28	4.28
Tryptophan	1.36	1.35	1.35
Arginine	5.99	5.95	5.97
Isoleucine	3.95	3.91	3.93
Leucine	6.95	6.94	6.94
Valine	5.11	5.07	5.09
Histidine	2.70	2.67	2.69
Phenylalanine	3.98	3.98	3.98

Whilst total lysine content is higher for solvent meal as shown in Table 4, reactive lysine is lower. The ratio of reactive/total lysine provides an indication of lysine loss resulting from heat processing damage. The data indicates that heat damage occurring in solvent meal is higher than that for expeller meal, this being 15% and 5% lysine loss respectively for solvent and expeller meals.

Table 4. *Total and reactive lysine content of expeller and solvent extracted canola meals (g/kg, as received)*

	Expeller	Solvent
Total lysine	19.53	21.17
Reactive lysine	18.44	18.05
Reactive lysine/Total lysine	0.945	0.853

Figures 1 and 2 show there is no relationship between reactive lysine and either crude protein or total lysine. This is in agreement with data generated within Stage 1, and confirms that variation in reactive lysine is more likely to be affected by other factors such as heat processing.

Figure 1. Scatter plot of Reactive lysine vs protein in canola meal

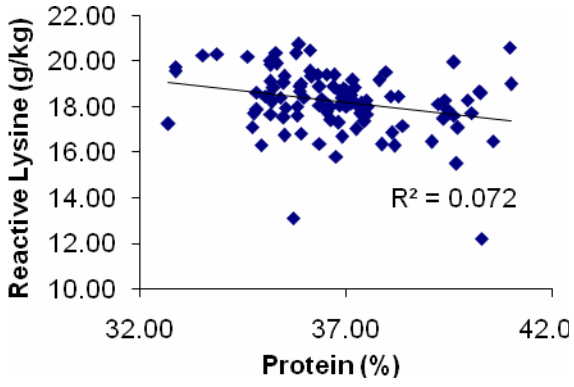
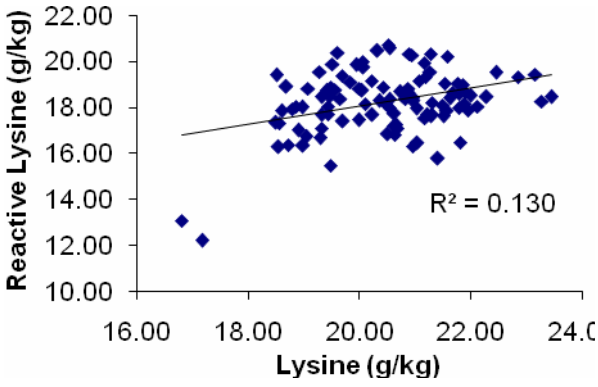


Figure 2. Scatter plot of Reactive lysine vs total lysine in canola meal



There was found to be no correlation between total lysine and reactive lysine as shown in Figure 3. Lysine loss, being the difference between total lysine and reactive lysine is shown in Figure 4 as the ratio of total to reactive lysine. It is seen that lysine loss ranges from nil (ratio of 1:1) in a small number of samples to a high of 30%.

Figure 3. Reactive lysine and total lysine for individual canola meal samples

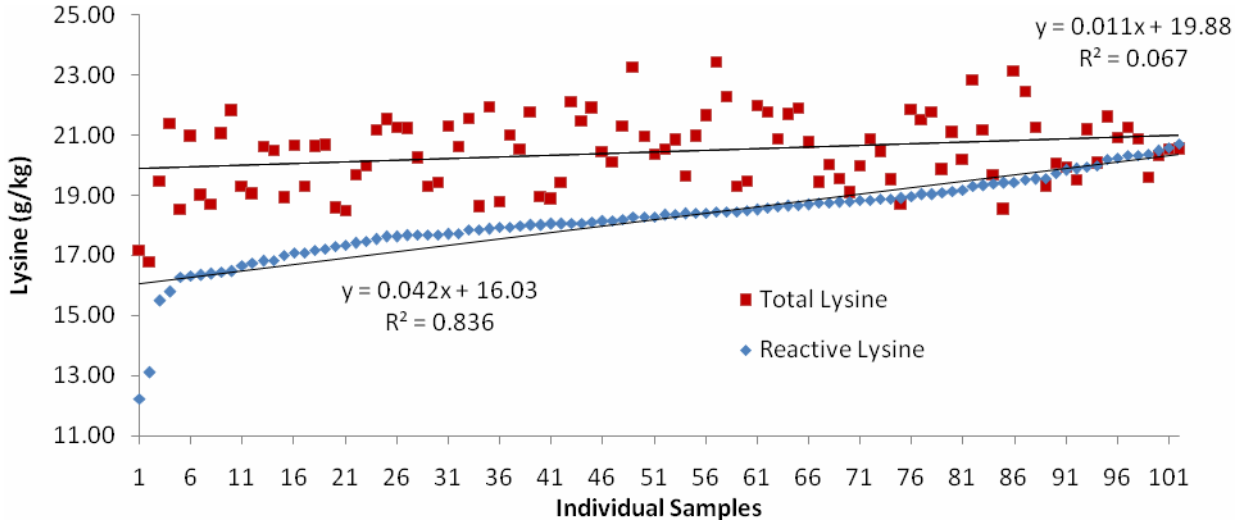
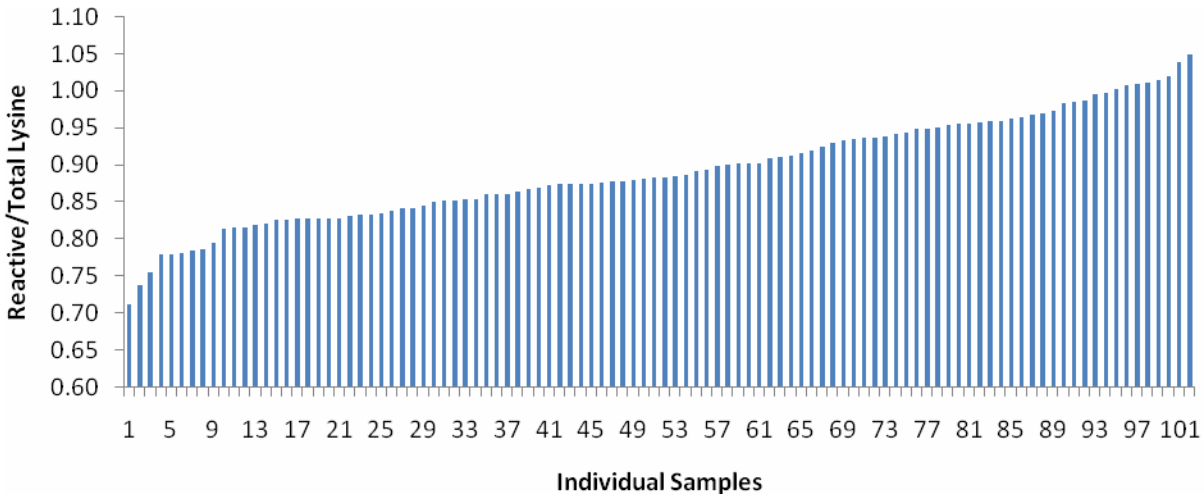


Figure 4. Ratio of reactive lysine to total lysine for individual canola meal samples



4.2 NIR Calibrations

An initial investigation looking at developing separate expeller and solvent meal calibrations confirmed that a combined calibration would provide a more robust calibration.

Calibration statistics are provided in Table 5 for total lysine, reactive lysine, reactive lysine/total lysine and reactive lysine/protein. The results obtained indicate that the NIR calibration predications explained 81% of the variation in total lysine and 76% of the variation

in reactive lysine. The RPD values provide a guide to the potential for the calibrations as predictors of total and reactive lysine. A value above 2.0 indicates the calibration is quantitative, whilst values 1.5 – 2.0 indicates that the calibration can distinguish between high and low results. Both the total lysine and reactive/total lysine calibrations are seen as being capable of differentiating between high and low results. Figures 5 and 6 show NIR predicted versus wet chemistry reactive lysine results. It is identified that the calibrations require further improvement to increase their commercial application for industry.

Table 5. NIR calibration statistics for reactive lysine, total lysine, reactive lysine/total lysine and reactive lysine/protein in canola meal

Description	N	Mean	SD	Min	Max	RSQ	SECV	1-VR	RPD
Total lysine (g/kg, as received)	99	20.43	1.18	16.91	23.96	0.81	0.69	0.66	1.72
Reactive lysine (g/kg, as received)	96	18.30	1.18	14.77	21.83	0.76	0.95	0.37	1.24
Reactive/Total lysine (g/g)	96	0.89	0.07	0.68	1.10	0.75	0.05	0.57	1.54
Reactive lysine/Protein (g/%)	96	5.06	0.49	3.60	6.51	0.87	0.26	0.71	1.85

SD, Standard deviation; RSQ, Square of correlation co-efficient R; SECV, Standard error or cross validation; 1-VR, Coefficient of determination for cross validation, RPD; Ratio of prediction to deviation (SD/SECV).

Figure 5. Scatter plot of reactive lysine (g/kg) NIR vs wet chemistry for all canola meal samples ground prior to NIR scanning

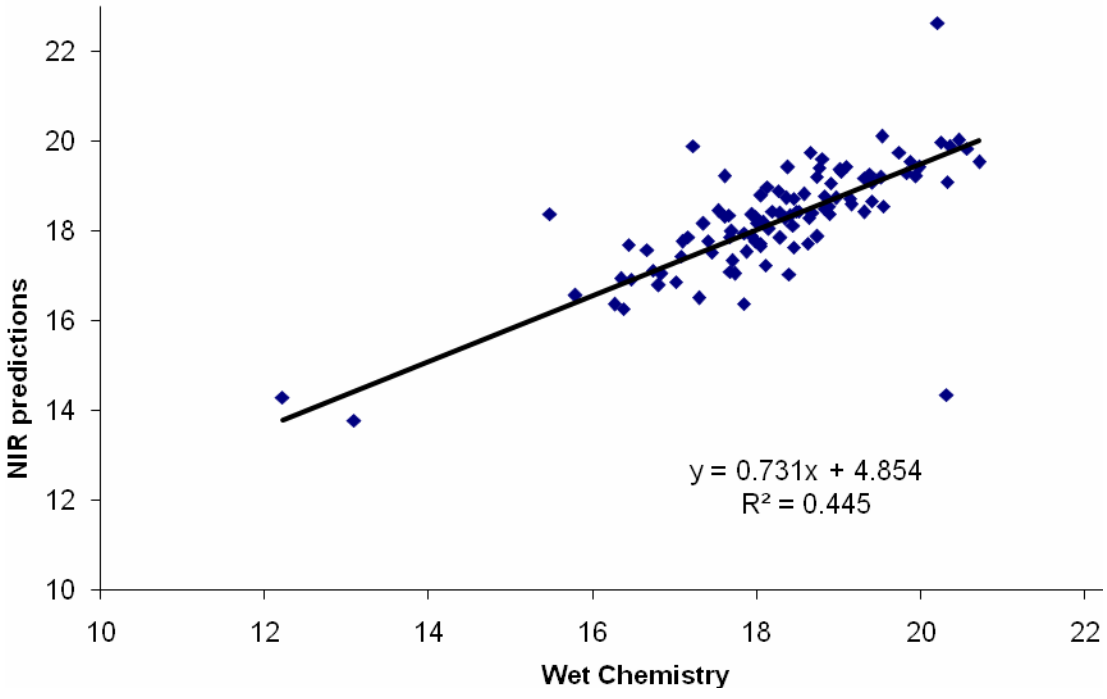
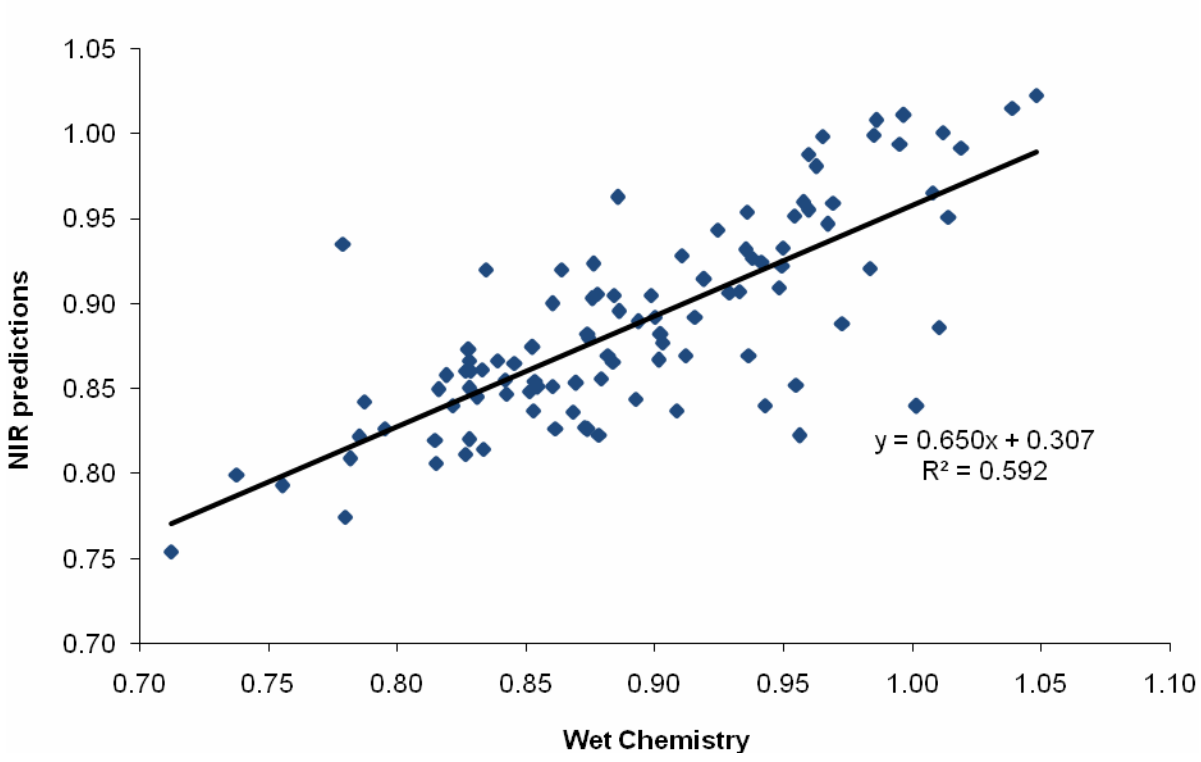


Figure 6. Scatter plot of reactive lysine/total lysine NIR vs wet chemistry for all canola meal samples



The potential improvement in NIR calibrations through the grinding of samples prior to NIR scanning was assessed. Calibrations were developed using samples that were either unground (as received) or ground (0.5mm screen). Calibration statistics for reactive lysine is shown in Table 6. It was found that grinding made a small improvement in the calibration statistics.

Table 6. NIR calibration statistics for reactive lysine using unground or ground canola meal

Description	N	Mean	SD	Min	Max	RSQ	SECV	1-VR	RPD
Unground (g/kg, as received)	96	18.30	1.18	14.77	21.83	0.76	0.95	0.37	1.24
Ground (g/kg, as received)	95	18.30	1.19	14.73	21.87	0.73	0.93	0.40	1.28

SD, Standard deviation; RSQ, Square of correlation co-efficient R; SECV, Standard error or cross validation; 1-VR, Coefficient of determination for cross validation, RPD; Ratio of prediction to deviation (SD/SECV).

Validation of each calibration was attempted using three subsets of the samples collected. Due to the limited number of samples included within each calibration, the removal of samples from the calibration affected the calibrations accuracy and capacity to obtain statistical validation.

5 Discussion and Recommendations

When protein materials are heat treated, chemical complexes may form between amino acids and carbohydrates. Although these complexes may be digested and absorbed, the amino acid component is unavailable to the animal for metabolism. Lysine is known to be more affected by heat processing than other amino acids. The premise for this project is that reactive lysine is an accurate reflection of lysine availability, this relationship being investigated by Rutherford (1997) and van Barneveld (2001).

Using a wet chemistry based reactive lysine assay allows the development of NIR calibrations that reflect the effects of heat processing on canola meal. When comparing reactive lysine content between samples, it needs to be done relative to total lysine so that the effect of heat damage through lysine loss can be demonstrated. Using the proportion of reactive to total lysine provides a measure of the amount of total lysine that is “available” to the animal. Reactive/total lysine for canola meal ranged from 0.68 to 1.0. The data would indicate that there were some canola meal samples that had no measured lysine loss due to heat damage and would be highly digestible in pig rations. It is of note that the level of lysine loss within this set of canola meal samples was less than that reported within stage 1. Whilst this may have been due to changes in crushing plant processing to reduce excessive use of heat during processing, there would also appear to have been a higher recovery of reactive lysine from Stage 2 sample analysis. A response from Massey University has indicated that less than adequate sample preparation may have resulted in lower reactive lysine results from Stage 1. Further work is being completed by Massey University to verify results, with additional data being used to improve the calibrations.

Data from this project, obtained in both Stage 1 and Stage 2, identifies that reactive lysine values are not correlated with total lysine. This finding is in agreement with the work completed by van Barneveld (2001) looking at canola meal and milk powder. Recent work by Redman et al (2008a and 2008b) looking at commercial samples of soybean meal and dried distillers grains with solubles has found that reactive lysine retains a set differences to total lysine, and they have concluded that total lysine is an equal measure of protein quality and potential heat damage. In contrast the results from this project support the use of reactive lysine as a better measure of processing damage for canola meal.

Limitations in the development of the NIR calibrations occurred due to the relative high cost of reactive lysine analyses. The reactive lysine analysis is a difficult assay to perform with a within duplicate CV of 5%. Inclusion of more samples with greater variability of lysine loss due to heat damage is required to increase the calibrations accuracy.

The range of samples for this project were obtained from commercial crushing plants and these encompass the material being supplied within the marketplace. It is recognised that the NIR reactive lysine calibration developed by van Barneveld (2001) as well as reactive lysine work completed by Redshaw et al (2008a and 2008b) utilised a combination of commercial samples and material subject to higher levels of heat from oven incubation or autoclaving. It was the view of the project team that whilst inclusion of more extreme heat damaged samples would provide a more robust calibration with improved NIR statistics, the calibration needed to be based upon real world samples. Based upon the less than desired NIR statistics, the inclusion of more heat damaged samples needs to be investigated to improve the calibrations accuracy.

Whilst the calibration has limitations in its accuracy, it is anticipated that this will be improved through the incorporation of additional samples being analysed at Massey University. When

this is completed it is recommended that the calibrations be made available on a research trial basis to selected industry participants to evaluate their usefulness. This is anticipated to include oilseed crushers, feed mills and larger pig producers with access to NIR machines.

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