Further development of a reactive lysine NIR calibration for soybean meal
4B-110

Report prepared for the Co-operative Research Centre for High Integrity Australian Pork

By

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Executive Summary

A previous study (CRC for an Internationally Competitive Pork Industry project - 1B-112) determined that with imported soybean meals there was a 27% of cross-shipment variation and a 13% within-shipment variation in reactive lysine content, and demonstrated that total lysine content should not be used for accurate prediction of reactive lysine content. Based on the data set obtained from 216 soybean meal samples a NIR calibration was establish with the standard error of cross validation for reactive lysine of ±0.82 g/kg (as is basis, with an R^2 of 0.86). However, validation statistics indicated that this calibration is not accurate enough for quality control as the RDP (ratio of prediction to deviation, SD/SECV) value was less than 2, whereas it needs to be higher than 3 to be a robust calibration.

In the current study we collected, heat treated, analysed, and included 68 soybean meal samples and 25 soy protein concentrate samples into an updated calibration. The updated calibration provides the prediction of total and reactive lysine with the standard error of cross validations of ±1.02 g/kg and ±0.96 g/kg (as is basis), respectively, with R^2 of 0.94 and 0.95. These values mean that the total and reactive lysine contents of unknown soybean samples should be predicted with 95% confidence to be within 2.04 and 1.92 g/kg of the actual value. As indicative in the RPD values of 3.35 and 3.94 for total and reactive lysine calibration, respectively, the new NIR calibration offers a robust prediction for bioavailable lysine content in soybean meal and soy protein concentrate. In addition, the new calibration has the ability to predict apparent, standardized and true ileal digestible total and reactive lysine contents in soybean meal and soy protein concentrate with similar accuracy.

The two major achievements accomplished in this project are:

1. the new soybean meal NIR calibration now has robust prediction accuracy and can be used for prediction of total and ileal digestible bioavailable lysine contents.
2. the calibration can also be used for soy protein concentrate.

This calibration can be applied in commercial feed mills, nutrition labs and SBM trading companies to accurately evaluate the quality of SBM. Furthermore, use of predicted standardized reactive lysine content for diet formulation will improve nitrogen utilisation efficiency by the Australian Pork industry. In addition, use of this technology in the Australian pig industry will reduce nitrogen excretion into the environment.

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1. Introduction

The previous project "1B-112 Quantification of the variability in the amino acid and reactive lysine content of soybean meal and development of a NIR calibration for rapid prediction of reactive lysine content" established a NIR calibration for reactive lysine in soybean meal samples. In this project 210 samples were collected and used for establishment of the NIR prediction calibration for reactive lysine content. The established calibration, however, needs to be updated with more soybean meal samples to improve the accuracy of reactive lysine prediction (currently R^2 = 0.86, SECV = 0.82 g/kg). One major problem with the previous sampling practice was that the importation of low quality soybean meal samples from overseas for *in vitro* analysis was impossible due to quarantine restrictions, and several batches of samples were destroyed by AQIS. Also due to the relatively narrow range of reactive lysine content in soybean meal samples compared with DGGS and canola meal, more samples (especially low quality soybean meals) need to be collected to strength the predictability. Therefore, the overall aim of this project was to strength the predictability of the existing soybean meal reactive lysine NIR calibration to the level that is applicable for robust and reliable measure of reactive lysine content.

2. Methodology

As importation of low quality soybean meal samples could not be assured due to quarantine regulations, a decision was made to create heat damage from the selected existing samples to mimic the low quality soybean meal samples. In addition, it was also decided to include a number of soy protein concentrate samples without and with heat treatment as soy protein concentrate has similar colour and texture to soybean meal and would allow to further extend the range of reactive lysine content within the calibration. Based on this decision, 22 stored soybean meal samples containing a range of reactive lysine contents were selected for heat treatment (Table 1). The 22 selected samples were divided into 3 equal portions and randomly allocated to 3 different durations of heat treatments (either 5, 15, 25 min or 10, 20, 30 min) to create variable heat damage (Table 1). Also, soy protein concentrate samples from 6 consecutive importation batches were randomly allocated to 3 different heat treatments to create a range of heat damage (Table 1).

Heat treatment was done using an Atherton Horizon Sterilizer (A.E. Atherton & Son Pty Ltd., Victoria, Australia). A 1 kg soybean meal sample was packed in a cotton calico bag and placed over 500 mL of sterilised water (Figure 1). The heat treatment was initiated with a 6.5 min steam charge to bring the chamber temperature and pressure up to 134 °C and 217 Kpa, respectively. Then for the sterilising stage the chamber temperature and pressure remained as required (either 5, 10, 15, 20, 25 or 30 min and 217 Kpa). The sample was dried for 5 min at 110 °C and 14 Kpa before discharge from the chamber. After heat treatment, samples were air dried and a 50 gram representative subsample was tested for reactive lysine and amino acids. Details of amino acid and reactive lysine analysis were described in the previous report (Final report for project 1B-112). Remaining samples were scanned through a FOSS NIRS (FOSS XDS, Häganäs, Sweden) to obtain the spectrum.

One-way analysis of variance (ANOVA) was used to test statistical significance of reactive lysine content in SBM and soy protein concentrate samples. To examine the relationship between heat treatment and amino acid content in SBM and soy protein concentrate, a regression analysis was conducted.

Table 1 - Reactive lysine content of selected samples and random allocation of samples to create variable heat damage

Original sample ID	Origin	Reactive Lysine (g/kg)	Autoclave time at 134 °C		
			Treatment 1	Treatment 2	Treatment 3
1	USA	21.9	5	15	25
144	S. America	23.2	10	20	30
118	Argentina	23.9	5	15	25
106	Argentina	24.2	10	20	30
173	Brazil	24.4	5	15	25
105	Argentina	24.6	10	20	30
8	USA	24.7	5	15	25
7	USA	24.8	10	20	30
207	Brazil	24.9	5	15	25
48	China	25.0	10	20	30
86	Brazil	25.1	5	15	25
196	S. America	25.3	10	20	30
165	Brazil	25.4	5	15	25
94	Brazil	25.6	10	20	30
126	Brazil	25.7	5	15	25
129	Brazil	26.0	10	20	30
23	China	26.2	5	15	25
101	Brazil	26.4	10	20	30
122	USA	26.7	5	15	25
45	USA	27.2	10	20	30
33	India	27.7	5	15	25
30	India	30.1	10	20	30
286[1]	Brazil	37.8	10	20	30
287[1]	Brazil	39.2	5	15	25
288[1]	Brazil	36.1	10	20	30
289[1]	Brazil	37.4	5	15	25
290[1]	Brazil	36.7	10	20	30
291[1]	Brazil	36.3	5	15	25

[1]Soy protein concentrate. All other samples were soybean meal

Figure 1 - Soybean meal samples packed in a calico bag and placed over sterilized water before entry to the Atherton Sterilizer.

In the previous calibration, a total of 216 samples (209 soybean meal samples plus 7 heat treated reference samples) were used for establishment of the NIR calibration for soybean meal. In this project, a total of 93 samples (5 heat treated soybean meal samples used for an *in vivo* ileal digestibility experiment, 63 heat treated soybean meal samples, and 25 soy protein concentrate samples without and with heat treatments)were added into the existing data set to further improve accuracy of prediction. Therefore, a total of 309 samples were included in the new soybean meal NIR calibration.

3. Outcomes

3.1.Amino acid composition of soy protein concentrate

The reactive lysine and amino acid composition of soy protein concentrate samples collected over 6 importation batches are presented in Table 2. The mean total lysine content was 38.1 g/kg air-dry basis and the difference between total and reactive lysine content was less than 5%.

Table 2 - Reactive lysine and amino acid composition (g/kg, air-dry basis) of soy protein concentrate[1] samples collected over 6 consecutive importation batches.

Received	June 2012			September 2012				
Batch No.	1	2	3	1	2	3	Mean	SEM
tLys[2]	38.3	39.8	36.1	38.0	38.2	38.2	38.1	0.49
rLys[3]	37.8	39.2	36.1	37.4	36.6	36.3	37.2	0.49
rLys/tLys, %	98.7	98.6	100.0	98.4	96.0	95.0	97.8	0.77
Met	9.4	9.4	8.6	8.9	8.8	8.7	9.0	0.38
Cys[5]	10.0	10.4	9.4	10.2	10.4	10.0	10.1	0.15
Thr	26.0	27.1	26.0	26.6	26.4	26.3	26.4	0.17
Ile	28.9	31.4	29.5	29.6	30.2	30.4	30.0	0.36
Leu	48.5	52.0	49.0	49.7	50.2	50.6	50.0	0.51
Val	29.0	31.4	29.8	29.7	30.1	30.5	30.1	0.33
Asp	72.7	77.2	72.8	74.5	75.4	76.1	74.8	0.74
Glu	112	120	110	115	115	117	115	1.4

Received	June 2012			September 2012				
Batch No.	1	2	3	1	2	3	Mean	SEM
Ser	36.7	38.6	36.2	37.3	37.1	36.9	37.1	0.33
His	16.9	17.0	14.9	17.5	18.6	18.6	17.3	0.56
Gly	26.7	28.0	26.1	26.8	27.0	26.9	26.9	0.25
Ala	27.6	29.1	27.9	28.3	28.3	28.2	28.3	0.20
Arg	46.0	49.3	45.6	46.7	47.4	48.6	47.2	0.60
Tyr	23.3	25.1	23.2	24.2	25.2	25.5	24.4	0.41
Phe	32.2	35.0	33.5	33.0	33.3	33.7	33.5	0.38
Pro	37.4	39.4	36.4	37.3	37.6	37.7	37.7	0.41
Total AA[4]	622	660	616	633	639	644	636	6.5

[1]Soy protein concentrate was imported from Brazil and samples were collected and provided by C Pyke Pty Ltd. [2]tLys: total lysine. [3]rLys: reactive lysine. [4]Excluding tryptophan. [5]Reported cysteine includes cysteine and cystine and generally higher than cysteine determined conventional analysis (need caution for interpretation).

Cysteine was analysed in the same aliquot after acid hydrolysis and the value includes both cysteine and cystine. Average value was about 1.5 g/kg higher than cysteine analysed using conventional method. Therefore, caution is required when interpreting cysteine data in this report.

3.2. Effect of heat treatment on amino acid composition of soybean meal and soy protein concentrate

Increasing autoclaving time linearly decreased total and reactive lysine contents in soybean meals (Table 3). Arginine and cysteine also linearly decreased as extent of the heat treatment was increased. Regression analysis showed that total and reactive lysine decreased by 0.23 g/kg (constant 27.3, R^2 0.71, RSD 1.60, P<0.001) and 0.29 g/kg (constant 25.7, R^2 0.78, RSD 1.63, P<0.001), respectively, per every minute of heat treatment at 134 °C (Figure 2).

Table 3. Effect of heat treatment on amino acid composition of soybean meal

	Autoclave time, min at 134 °C								Probability[1]			
AA	0	5	10	15	20	25	30	SEM	ANOVA	Lin	Quad	Cub
tLys[2]	27.1	26.8	24.6	24.4	22.3	21.9	20.1	0.50	***	***	NS	NS
rLys[3]	25.4	24.6	23.1	21.9	19.8	18.4	16.9	0.52	***	***	NS	NS
rvLys[4]	1.7	2.2	1.5	2.6	2.6	3.5	3.2	0.43	**	***	NS	NS
rLys:tLys	94	92	94	90	89	84	84	1.7	***	***	NS	NS
Met	6.8	7.0	6.9	6.7	6.6	6.7	6.7	0.11	NS	*	NS	NS
Cys[5]	8.5	7.2	6.7	6.7	6.3	6.2	5.8	0.24	***	***	**	*
Thr	19.9	20.0	19.7	19.9	20.0	19.9	19.9	0.27	NS	NS	NS	NS
Ile	21.5	22.4	22.2	22.1	21.7	22.0	21.9	0.31	NS	NS	NS	NS
Leu	36.5	37.3	36.9	37.1	36.4	37.0	37.0	0.42	NS	NS	NS	NS
Val	21.7	23.1	22.8	22.9	22.5	22.8	22.6	0.23	***	**	***	**
Ala	20.3	20.7	20.5	20.6	20.2	20.4	20.3	0.20	NS	NS	NS	NS
Arg	34.4	34.8	33.4	32.8	32.3	30.9	29.6	0.52	***	***	NS	NS
Asp	55.0	55.9	55.3	55.6	54.2	54.9	54.6	0.67	NS	NS	NS	NS
Glu	84.3	86.7	85.8	86.5	84.6	85.8	85.5	1.21	NS	NS	NS	NS
Gly	19.8	20.6	20.3	20.6	19.9	20.3	20.1	0.24	*	NS	*	*
His	13.0	13.2	12.6	12.7	12.4	12.6	12.5	0.29	NS	*	NS	NS
Phe	25.0	24.7	24.5	24.4	23.9	24.0	24.0	0.40	NS	**	NS	NS
Pro	27.7	32.1	31.9	31.4	30.3	31.5	31.8	0.53	***	***	***	***

	Autoclave time, min at 134 °C								Probability[1]			
AA	0	5	10	15	20	25	30	SEM	ANOVA	Lin	Quad	Cub
Ser	26.8	26.9	26.4	26.7	26.0	26.2	26.1	0.36	NS	*	NS	NS
Tyr	17.7	19.4	19.0	19.2	18.5	18.9	18.7	0.30	***	**	***	**
Total AA[6]	493	452	445	446	434	440	437	5.5	***	***	***	**

[1]NS: not significant, *P<0.05, **P<0.01, ***P<0.001. [2]tLys: total lysine, [3]rLys: reactive lysine, [4]rvLys: reverted lysine, [5]Cysteine includes cysteine and cystine, [6]excluding tryptophan.

A similar response was observed in soy protein concentrate. Increasing autoclaving time linearly decreased total and reactive lysine contents in soy protein concentrate (Table 4). Regression analysis showed that total and reactive lysine decreased by 0.20 g/kg (constant 37.3, R^2 0.71, RSD 1.37, P<0.001) and 0.41 g/kg (constant 35.6, R^2 0.87, RSD 1.75, P<0.001), respectively, for every minute of heat treatment at 134 °C (Figure 3).

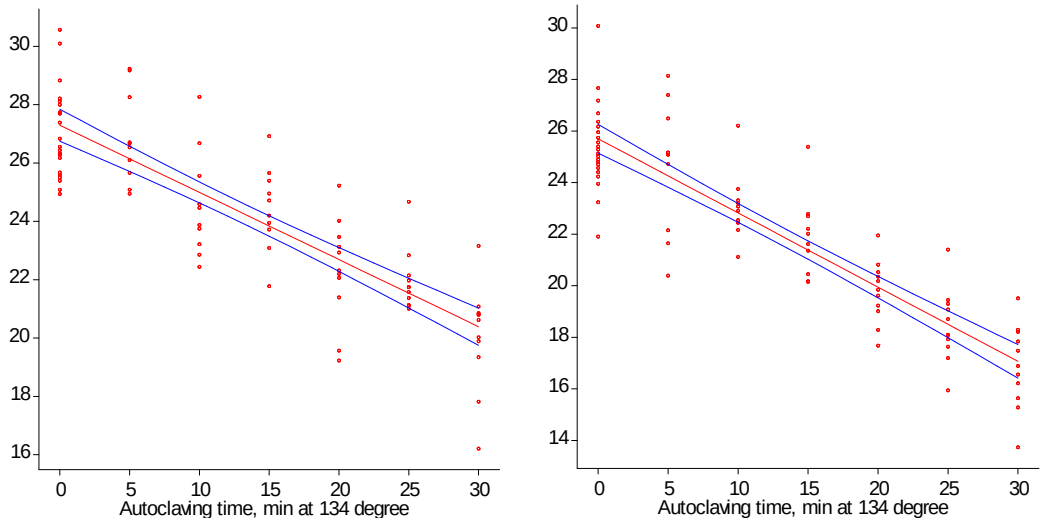


Figure 2 - Linear relationship between autoclaving time at 134 °C and lysine content in soybean meal (fitted line plot with 95% confidence interval).

Table 4 - Effect of heat treatment on amino acid composition of soy protein concentrate

	Autoclave time, min at 134 °C								Probability[1]			
AA	0	5	10	15	20	25	30	SEM	ANOVA	Lin	Quad	Cub
tLys[2]	37.8	36.1	33.9	34.7	32.8	33.0	31.7	0.77	***	***	NS	NS
rLys[3]	37.1	31.2	29.8	28.4	26.5	25.9	24.5	0.64	***	***	***	**
rvLys[4]	0.7	4.6	4.1	6.2	6.3	7.1	7.2	0.59	***	***	***	NS
rLys:tLys	98	87	88	82	81	79	77	1.6	***	***	*	NS
Met	8.8	9.0	8.9	8.9	8.9	9.1	8.7	0.27	NS	NS	NS	NS
Cys[5]	10.1	8.3	7.8	7.5	7.0	6.8	6.5	0.15	***	***	***	***
Thr	26.2	26.0	25.2	26.0	25.3	25.8	25.2	0.39	NS	NS	NS	NS
Ile	29.8	28.7	27.0	29.0	27.7	28.8	27.7	0.52	**	**	NS	*
Leu	49.5	48.2	46.1	48.3	46.7	48.1	46.6	0.85	*	*	NS	NS
Val	29.9	29.5	27.9	29.6	28.5	29.6	28.5	0.50	*	NS	NS	NS
Ala	27.9	26.7	25.7	26.8	26.1	26.6	26.0	0.46	**	**	*	NS
Arg	46.8	45.2	42.6	44.4	42.1	43.5	41.5	0.87	***	***	NS	NS
Asp	73.9	71.0	67.2	71.0	67.8	70.2	67.6	1.33	**	***	NS	NS
Glu	114	113	107	113	108	112	108	2.0	NS	*	NS	NS
Gly	26.7	25.4	24.3	25.5	24.7	25.2	24.5	0.40	***	***	*	*
His	16.5	15.1	12.7	15.1	12.5	14.6	12.8	1.22	NS	*	NS	NS
Phe	33.0	33.1	31.4	33.0	31.7	33.0	31.5	0.64	NS	NS	NS	NS
Pro	37.8	34.5	32.4	34.7	34.0	34.6	35.2	0.83	***	**	***	*
Ser	36.8	35.4	34.1	35.0	34.0	34.5	33.6	0.58	**	***	NS	NS
Tyr	24.3	21.7	20.9	21.8	21.2	22.1	21.4	0.39	***	***	***	***
Total AA[6]	624	616	584	612	587	605	584	13.6	NS	*	NS	NS

[1]NS: not significant, *P<0.05, **P<0.01, ***P<0.001. [2]tLys: total lysine, [3]rLys: reactive lysine, [4]rvLys: reverted lysine, [5]Cysteine includes cysteine and cystine, [6]excluding tryptophan.

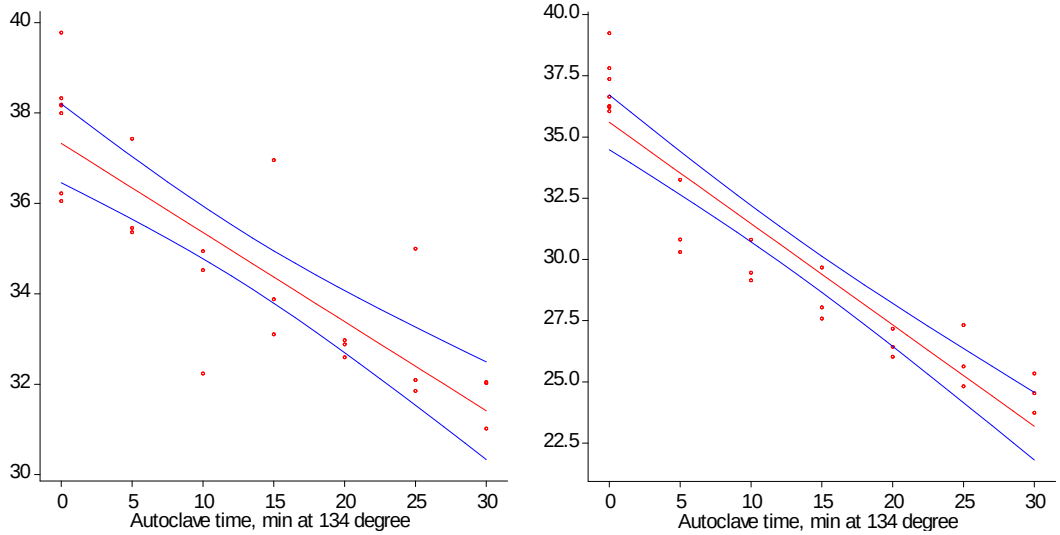


Figure 3 - Linear relationship between autoclaving time at 134 °C and lysine content in soy protein concentrate (fitted line plot with 95% confidence interval).

3.3. NIR calibration statistics for total and reactive lysine in soybean meal samples

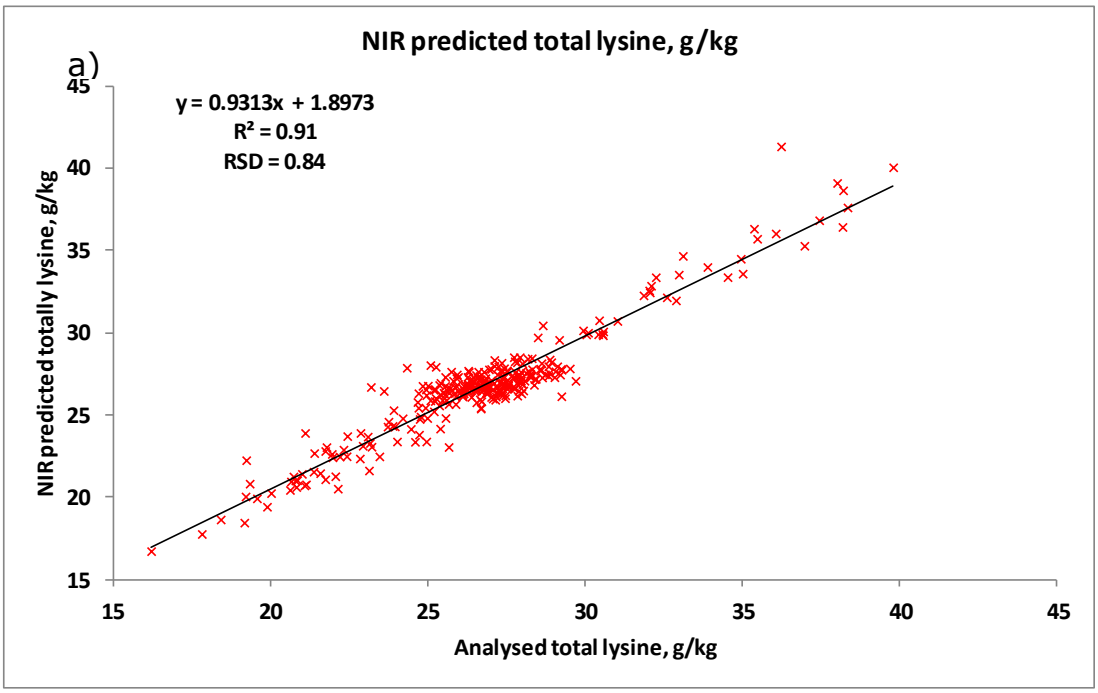
After adding the data from 93 samples including heat damaged soybean meal and soy protein concentrate samples, the existing calibration was recalibrated and cross validated. Soybean meal NIR calibration statistics for total, reactive, reverted lysine predictions and reactive over total lysine ratio prediction are presented in Table 5.

Table 5 - Soybean meal NIR calibration statistics for total lysine (tLys, g/kg), reactive lysine (rLys, g/kg), reverted lysine (rvLys, g/kg) and reactive over total lysine ratio (rLys:tLys, %).

Nutrient	*n*=	O[1]	Min	Max	Mean	SD[2]	SEC[3]	RSQ[4]	SECV[5]	1-VR[6]	RPD[7]
tLys	298	15	16.2	39.8	26.8	3.42	0.86	0.94	1.02	0.91	3.35
rLys	300	15	11.2	39.2	24.5	3.77	0.87	0.95	0.96	0.94	3.94
rvLysine	287	23	-0.4	7.7	2.3	1.63	0.89	0.70	0.97	0.65	1.69
rLys:tLys	299	15	60.8	100.4	91.5	6.54	3.42	0.73	3.67	0.68	1.78

[1]O: number of outliers removed; [2]SD: Standard deviation; [3]SEC: Standard error of calibration; [4]RSQ: The fraction of the explained variance in the calibrations; [5]SECV: Standard error of cross validation; [6]1-VER: Fraction of explained variance in the cross validation; [7]RPD: Ratio of prediction to deviation (SD/SECV).

The calibration statistics represent quality of prediction. Ideally RSQ and fraction of explained variance in the cross validation (1-VR) should be close to 1 and standard error of cross validation (SECV) should be as small as possible. One of the measures that indicate quality of calibration is the ratio of prediction to deviation (RPD, SD/SECV). A RPD value of less than 2 is considered as not providing an improved prediction compared to using a mean value. A RPD value higher than 3 is required if a NIR calibration for screening is to be classed as satisfactory (Andueza et al., 2011). Compared with the initial calibration (Project 1B-112 final report), the updated calibration improved RPD values for total lysine and reactive lysine from 1.59 and 2.32 to 3.35 and 3.94, respectively. However, the RPD values for reverted lysine and the reactive lysine:total lysine ratio were less than 2, indicating the current calibration will not provide accurate predictions for these measurements. Scatter plots for analysed versus predicted total and reactive lysine in soybean meal samples are presented in Figure 4.



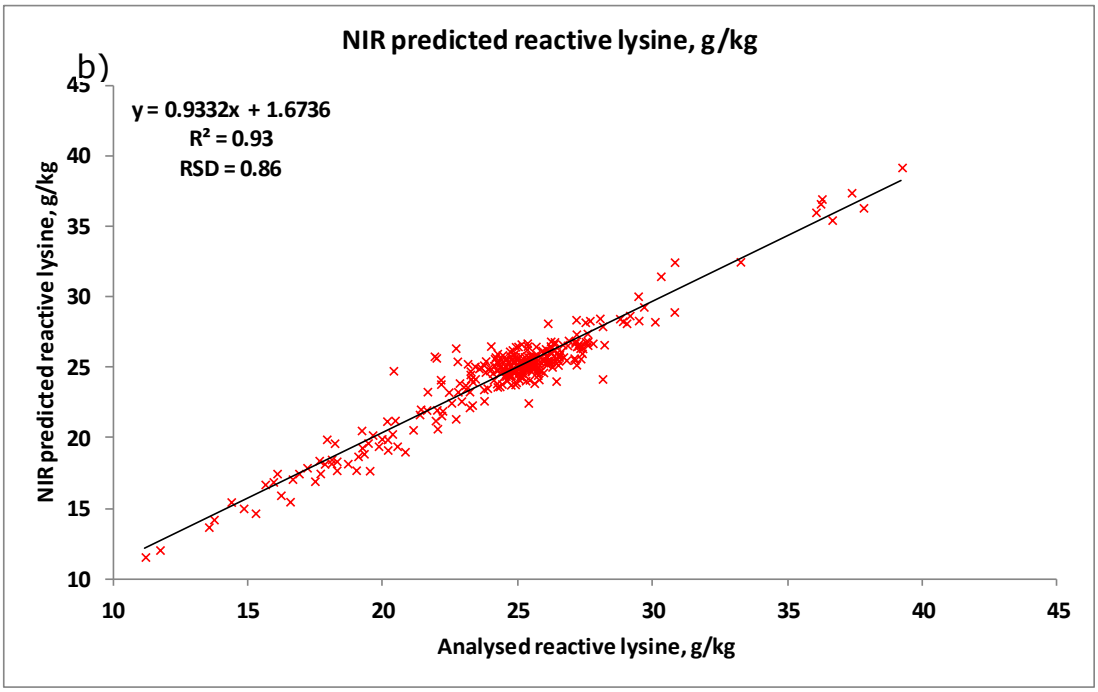


Figure 4 - Scatter plot of analysed vs NIR predicted total lysine (a) and reactive lysine (b) contents in soybean meal samples

Another important validation factor is standard error of prediction (SEP), which is monitoring the difference between standard error of calibration (SEC) and SECV. To avoid over-lifting errors, the difference between SEC and SECV should not be higher than 20% of SEC (Andueza et al., 2011, Payne et al., 2011). Both total lysine and reactive lysine calibration had less than 20% of SEP. Based on the validation statistics, the current soybean meal NIR calibration: (1) significantly improved prediction accuracy compared with the previous calibration; (2) was able to accurately predict total and reactive lysine contents in both soybean meal and soy protein concentrate, and (3) is not suitable for prediction of reverted lysine. Calibration statistics for the other amino acids and chemical composition are presented in Appendix 1.

The other amino acids that are significantly damaged by heat treatment were arginine and cysteine (Table 4). Calibration statistics for arginine indicates that arginine can be accurately predicted (R^2 = 0.95, RPD = 3.89), however, calibration for cysteine does not provide accurate prediction (R^2 = 0.76, RPD = 1.79, see Appendix 1).

Scatter plots for analysed and predicted arginine and cysteine are presented in Figure 5. The weak predictability of cysteine is probably due to use of a modified method in this study that detected both cysteine and cystine in the sample. Analysis of cysteine using the conventional extraction method may improve prediction accuracy and further upgrade project should aim for cysteine calibration as cysteine is an important essential amino acid that affects protein utilization efficiency.

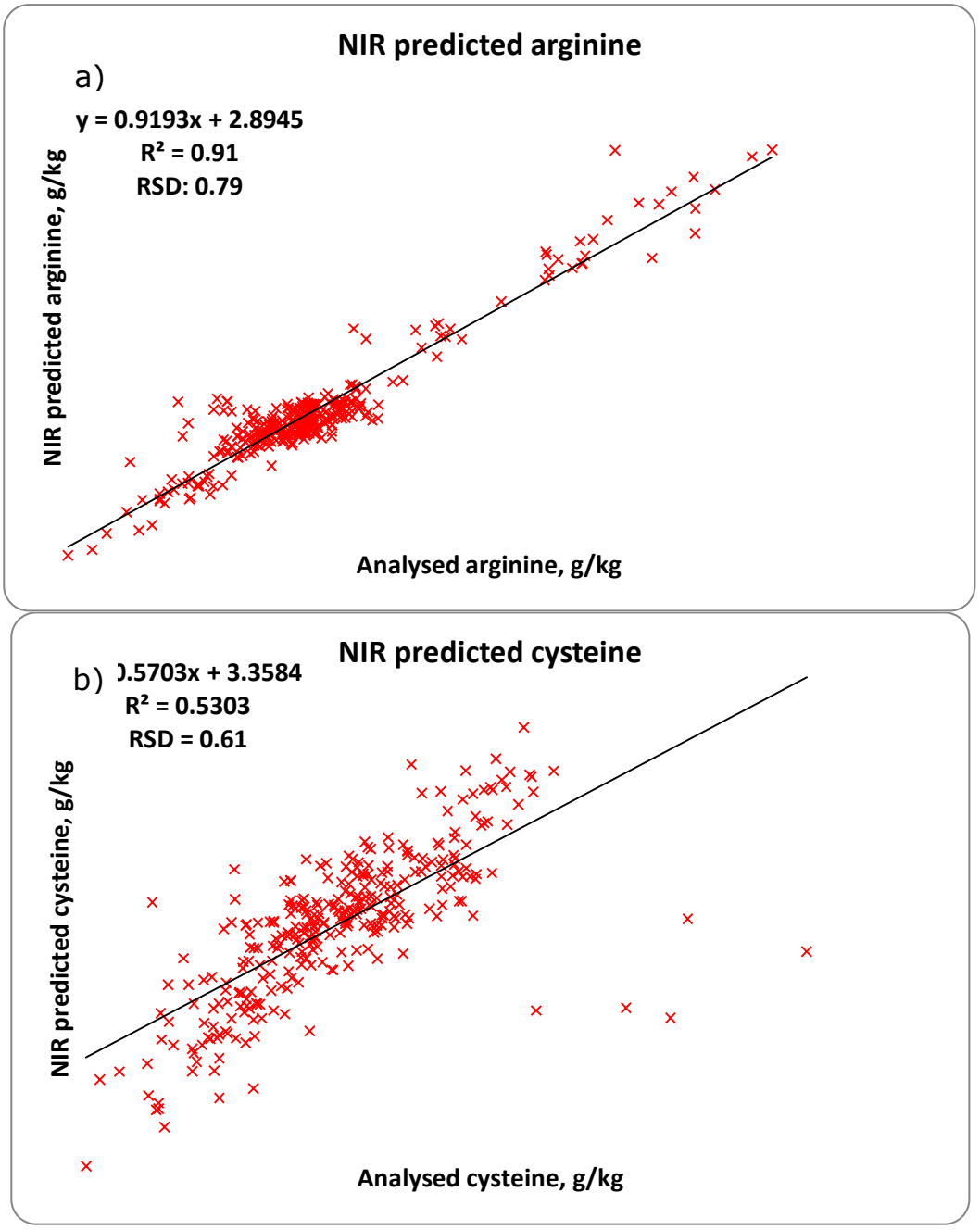


Figure 5 - Scatter plot of analysed vs NIR predicted arginine (a) and cysteine (b) contents in soybean meal samples. Reported cysteine includes cysteine and cystine and generally higher than cysteine determined conventional analysis (need caution for interpretation).

3.4. NIR calibration statistics for apparent, standardized and true ileal digestible total and reactive lysine contents

Not all reactive lysine present in a soybean meal is digested and absorbed in the small intestine of pigs. Therefore, ideally the calibration should be able to predict for apparent ileal digestible (AID) total and reactive lysine contents, which are the total and reactive lysines that are available for protein deposition. Standardised (SID) and true ileal digestible (TID) total lysine and reactive lysine are the values corrected for basal endogenous amino acid loss after feeding protein-free diet and corrected for specific endogenous amino acid loss after feeding theoretically 100% digestible amino acids. Therefore, another aim of the project was to improve predictability of apparent (AID), standardized (SID), true ileal digestible (TID) total lysine and reactive lysine contents in the soybean meal and soy protein concentrate. The six linear regression equations developed from the previous *in vivo* experiment (Project 1B-112) where apparent,

standardized and true ileal digestibilities were determined in growing pigs after feeding a range of heat damaged soybean meal are presented in Table 6 (Kim et al., 2012).

Table 6 - Linear regression equations for prediction of apparent (AID), standardised (SID) and true ileal digestible (TID) total lysine (tLYS) and reactive lysine (rLYS) contents from total and reactive lysine contents, respectively, in soybean meal (after Kim et al., 2012).

	Intercept	Slope	RSD[1]	R^2	*P*=[2]
AID tLYS, g/kg as-fed	-8.37	1.2002	0.810	0.95	***
SID tLYS, g/kg as-fed	-7.88	1.2126	0.810	0.95	***
TID tLYS, g/kg as-fed	-7.62	1.2192	0.811	0.95	***
AID rLYS, g/kg as-fed	-2.841	1.0626	0.392	0.99	***
SID rLYS, g/kg as-fed	-2.440	1.0666	0.393	0.99	***
TID rLYS, g/kg as-fed	-2.193	1.0691	0.393	0.99	***

[1]RSD: Residual standard deviation.
[2]***P < 0.001

The regressed digestibility values from the total and reactive lysine contents in soybean meal and soy protein concentrate were then calibrated for prediction of ileal digestible total and reactive lysine contents. Calibration statistics for prediction of ileal digestible total and reactive lysine are presented in Table 7. All the prediction parameters showed a RPD of higher than 3 and SECV were less than 20% higher than SEC. Therefore, validation statistics indicate that the new NIR calibration provides accurate predictions for AID, SID and TID total and reactive lysine contents in soybean meal and soy protein concentrate. Scatter plots for regressed versus predicted AID, SID and TID total and reactive lysine in soybean meal samples are presented in Figures 6, 7 and 8.

Table 7 - Soybean meal NIR calibration statistics for apparent (AID), standardized (SID) and true ileal digestible (TID) total lysine (tLys) and reactive lysine (rLys) content (g/kg), reverted lysine (rvLys) and reactive over total lysine ratio (rLys:tLys).

Nutrient	*n*=	O[1]	Min	Max	Mean	SD[2]	SEC[3]	RSQ[4]	SECV[5]	1-VR[6]	RPD[7]
AID tLys[8]	299	15	11.1	39.4	23.8	4.12	1.05	0.94	1.23	0.91	3.36
AID rLys[9]	300	14	9.0	38.9	23.2	4.02	0.93	0.95	1.03	0.93	3.89
SID tLys[10]	298	17	11.8	40.3	24.6	4.13	1.05	0.94	1.24	0.91	3.35
SID rLys[11]	300	15	9.5	39.4	23.7	4.02	0.93	0.95	1.02	0.94	3.94
TID tLys[12]	298	16	11.9	40.5	24.8	4.15	1.05	0.94	1.23	0.91	3.38
TID rLys[13]	300	14	9.6	39.5	23.8	4.02	0.93	0.95	1.03	0.93	3.89

[1]O: number of outliers removed; [2]SD: Standard deviation; [3]SEC: Standard error of calibration; [4]RSQ: The fraction of the explained variance in the calibrations; [5]SECV: Standard error of cross validation; [6]1-VER: Fraction of explained variance in the cross validation; [7]RPD: Ratio of prediction to deviation (SD/SECV); [8]ADI tLys: Apparent ileal digestible total lysine; [9]AID rLys: Apparent ileal digestible reactive lysine; [10]SID tLys: Standardised ileal digestible total lysine; [11]SID rLys: Standardised ileal digestible reactive lysine; [12]TID tLys: True ileal digestible total lysine; [13]TID rLys: True ileal digestible reactive lysine;.

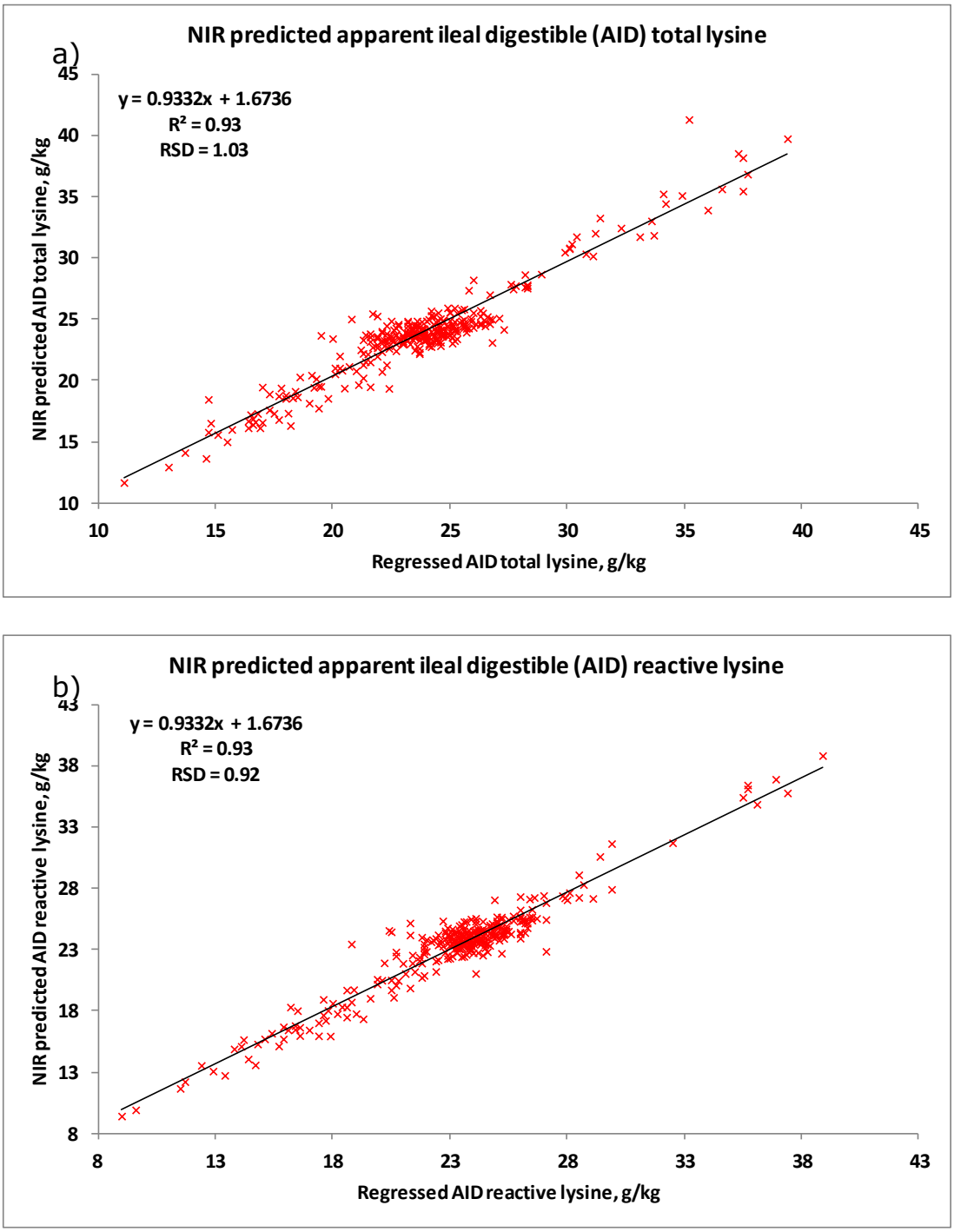


Figure 6 - Scatter plot of regressed vs. NIR predicted apparent ileal digestible (AID) total lysine (a) and reactive lysine (b) contents in soybean meal samples.

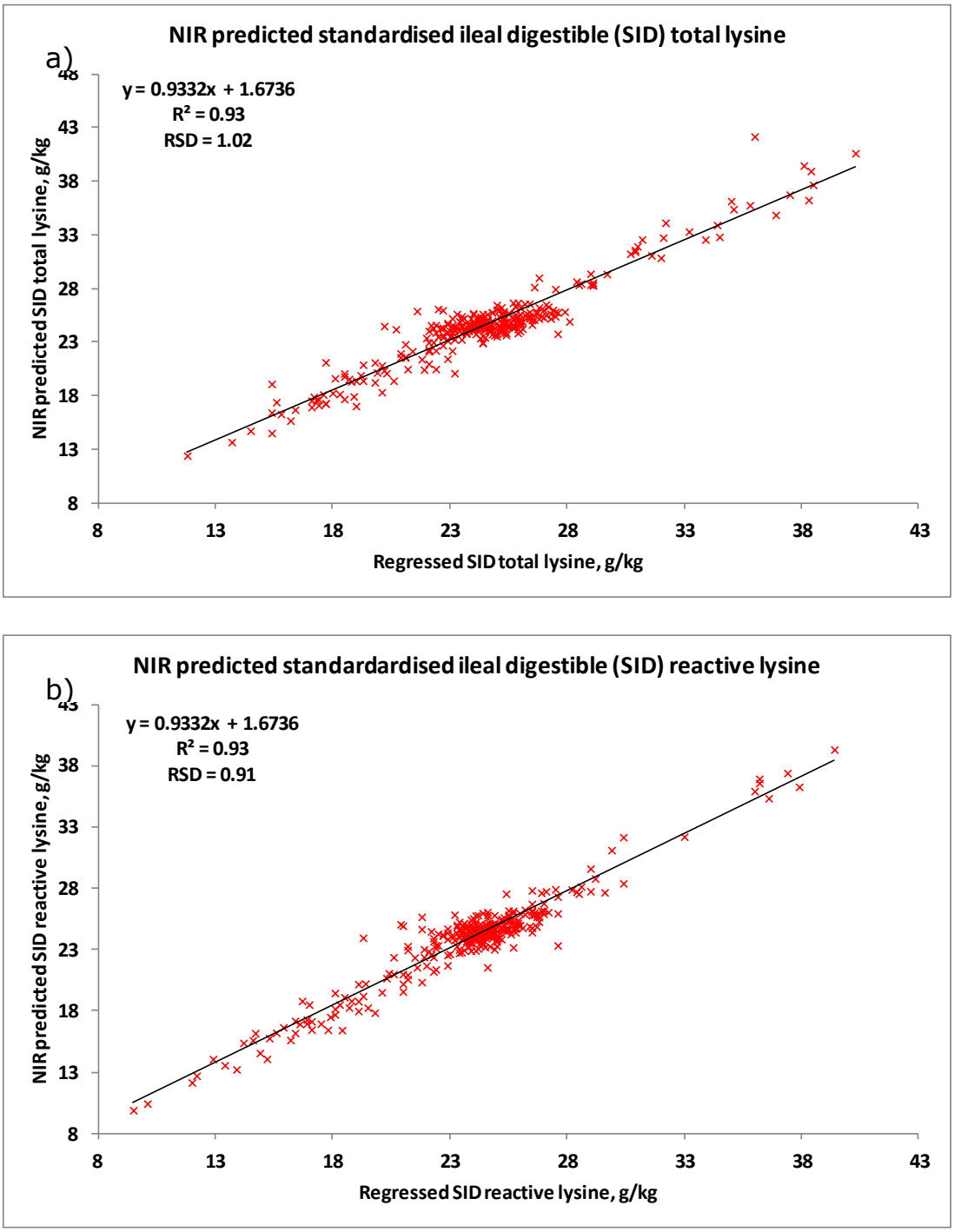


Figure 7 - Scatter plot of regressed vs. NIR predicted standardised ileal digestible (SID) total lysine (a) and reactive lysine (b) contents in soybean meal samples.

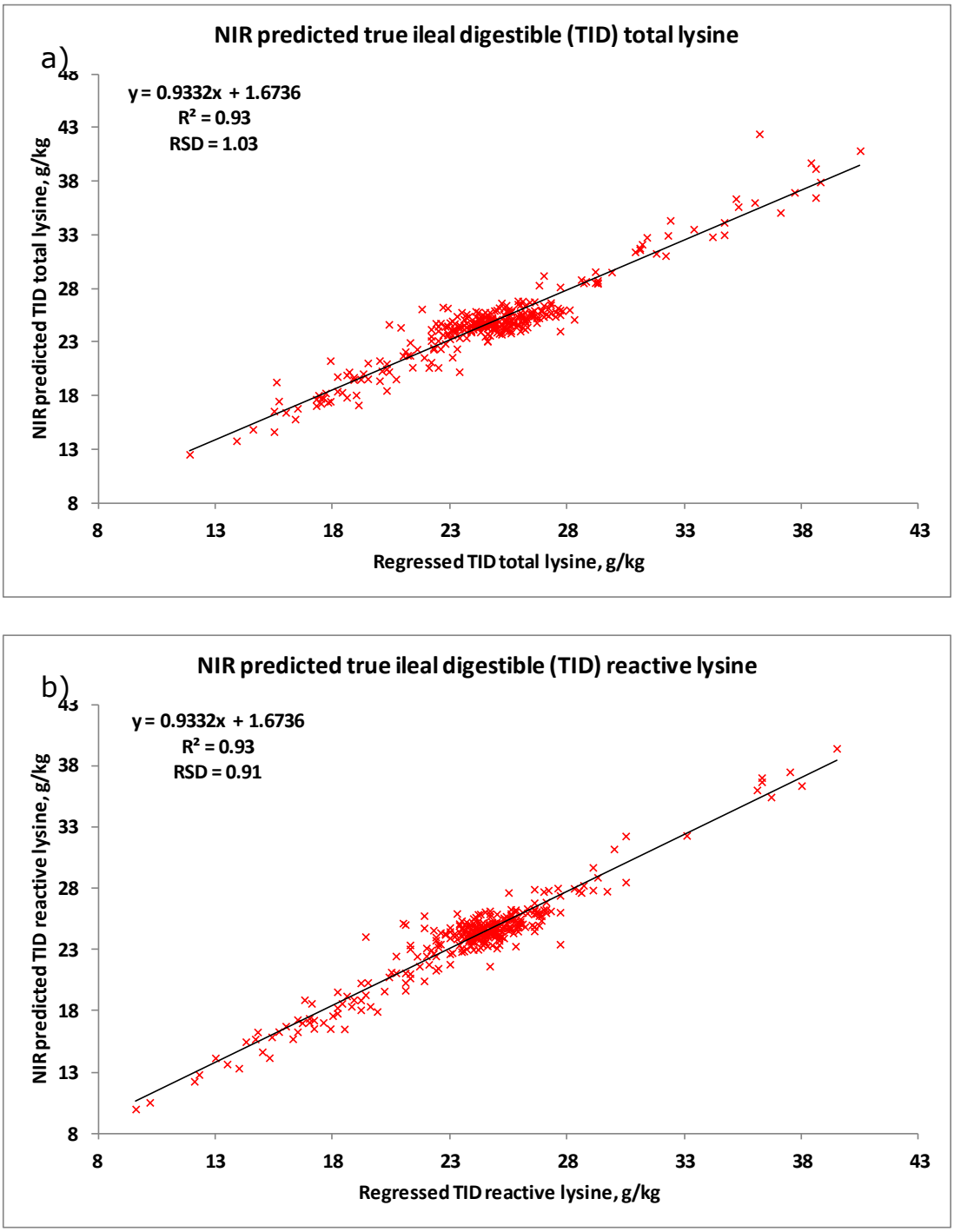


Figure 8 - Scatter plot of regressed vs. NIR predicted true ileal digestible (TID) total lysine (a) and reactive lysine (b) contents in soybean meal samples.

4. Application of Research

A previous study (1B-112) determined that with imported soybean meals there was a 27% cross-shipment variation and a 13% within-shipment variation in reactive lysine content, and demonstrated that total lysine content cannot be used for accurate prediction of reactive lysine content. Based on the data set obtained from 216 soybean meal samples a NIR calibration was established with the standard error of cross validation for reactive lysine of ±0.82 g/kg (as is basis, with an R^2 of 0.86). However, validation statistics indicated that the calibration is not accurate enough for quality control as the RDP value was less than 2, whereas it should be higher than 3 to be a robust calibration. In the current study we collected, heat treated, analysed, and included 68 soybean meal

samples and 25 soy protein concentrate samples into an updated calibration. The updated calibration provides prediction of total and reactive lysine with the standard error of cross validations of ±1.02 g/kg and ±0.96 g/kg (as is basis), respectively, with R^2 of 0.94 and 0.95. According to these SECV values any sample should be predicted with a 95% probability certainty of ± 2.04 g/kg and ± 1.92 g/kg respectively for total and reactive lysine.

Standard errors for the total and reactive lysine measured values were 0.197 g/kg and 0.213 g/kg, respectively. These SE values of measurement suggest that accuracy of NIR prediction of both lysine and reactive lysine could be improved with the addition of more samples, with SECV values approximating 0.5 g/kg being possible.

As indicative in the RPD values of 3.35 and 3.94 for total and reactive lysine calibration, respectively, the new NIR calibration offers a robust prediction for bioavailable lysine contents in the soybean meal and soy protein concentrate. In addition, the new calibration has the ability to predict apparent, standardized and true ileal digestible total and reactive lysine contents in soybean meal and soy protein concentrate with a similar accuracy.

These calibrations can be applied in commercial feed mills, nutrition labs and SBM trading companies to accurately evaluate the quality of SBM. Furthermore, use of predicted standardized reactive lysine content for diet formulation will improve nitrogen utilisation efficiency by the Australian Pork industry. In addition, use of this technology in the Australian pig industry will reduce nitrogen excretion into the environment.

5. Conclusion

The project established a robust NIR calibration for prediction of reactive lysine content and ileal digestible reactive lysine content with ±0.96 g/kg and ±1.02 g/kg (as is basis) standard error of cross validations for reactive lysine and standardized ileal digestible reactive lysine, respectively. These values mean that the total and reactive lysine contents of unknown soybean samples should be predicted with 95% confidence to be within 2.04 and 1.92 g/kg of the actual value. Use of the predicted reactive lysine content in feed formulation will significantly improve nitrogen retention efficiency in the Australian Pork industry.

6. Limitations/Risks

The established calibration provides robust prediction for total lysine, reactive lysine, ileal digestible total lysine, and ileal digestible reactive lysine content in the soybean meal and soy protein concentrate samples. Predicted values for other amino acids and chemical composition with RPD values less than 2 should be used with caution. The chemical composition with less than 2 RPD values include reverted lysine, total:reactive lysine ratio, cysteine, histamine, crude protein, crude fat, neutral detergent fibre and lignin.

7. Recommendations

As a result of the outcomes in this study the following recommendations have been made:

1. The total and reactive lysine and standardised ileal reactive lysine calibrations should be added to the AusScan suite of calibrations.
2. Nutritionists and feed mills should use predicted standardized ileal digestible reactive lysine content for formulation of pig diets to improve nitrogen retention efficiency.
3. Further improvement of the calibration is recommended to improve accuracy of the prediction, particularly for cysteine which has been shown be significantly depressed by heat treatment.

8. References

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The reactive lysine assay was developed and conducted by Dr Gerard Smith and Mr Mal McGrath at the Animal Health Laboratories, DAFWA.

The NIR calibrations have been developed by Dr Troy Adriansz at Grains and Product Innovation, DAFWA.

Heat treatment of soybean meal samples was done by Mrs Julia Carson at the Animal Health Lab, DAFWA.

Handling and processing of soybean meal samples was done by Mr Matthew Langridge at DAFWA.

Appendix 1 - Soy bean meal NIR calibration statistics

Nutrient	*n*=	O[1]	Min	Max	Mean	SD[2]	SEC[3]	RSQ[4]	SECV[5]	1-VR[6]	RPD[7]
tLys	298	15	16.2	39.8	26.8	3.42	0.86	0.94	1.02	0.91	3.35
rLys	300	15	11.2	39.2	24.5	3.77	0.87	0.95	0.96	0.94	3.94
RvLys	287	23	-0.4	7.7	2.3	1.63	0.89	0.70	0.97	0.65	1.69
rLys:tLys	299	15	60.8	100.4	91.5	6.54	3.42	0.73	3.67	0.68	1.78
AID tLys[8]	299	15	11.1	39.4	23.8	4.12	1.05	0.94	1.23	0.91	3.36
AID rLys[9]	300	14	9.0	38.9	23.2	4.02	0.93	0.95	1.03	0.93	3.89
SID tLys[10]	298	17	11.8	40.3	24.6	4.13	1.05	0.94	1.24	0.91	3.35
SID rLys[11]	300	15	9.5	39.4	23.7	4.02	0.93	0.95	1.02	0.94	3.94
TID tLys[12]	298	16	11.9	40.5	24.8	4.15	1.05	0.94	1.23	0.91	3.38
TID rLys[13]	300	14	9.6	39.5	23.8	4.02	0.93	0.95	1.03	0.93	3.89
Met	286	31	5.6	9.5	6.9	0.67	0.28	0.83	0.31	0.79	2.17
Cys	298	14	4.6	10.8	0.6	1.20	0.59	0.76	0.67	0.69	1.79
Thr	286	31	17.4	27.1	20.3	1.87	0.59	0.90	0.67	0.88	2.80
Ile	301	13	18.3	31.4	22.0	2.26	0.87	0.85	0.91	0.84	2.50
Leu	285	32	32.0	52.0	37.5	3.49	0.98	0.92	1.19	0.89	2.94
Val	299	13	19.7	31.4	22.4	2.23	0.66	0.91	0.72	0.90	3.12
Tyr	289	31	15.5	25.1	18.1	1.54	0.51	0.89	0.58	0.86	2.67
Phe	286	31	21.9	35.0	25.1	2.61	0.95	0.87	1.07	0.83	2.44
Ser	289	28	22.4	38.6	27.1	2.77	0.81	0.92	0.91	0.89	3.06
His	298	17	9.4	18.6	12.8	1.38	0.87	0.61	0.94	0.54	1.47
Gly	294	22	17.8	27.0	20.2	1.76	0.49	0.92	0.58	0.89	3.02
Ala	289	26	18.7	29.1	20.8	1.92	0.41	0.95	0.48	0.94	3.99
Asp	292	24	48.1	77.2	55.9	5.04	1.80	0.87	2.03	0.84	2.48
Glu	296	23	72.3	120.0	86.3	8.64	2.58	0.91	2.80	0.90	3.09
Arg	289	29	26.2	49.3	34.3	3.44	0.76	0.95	0.89	0.94	3.89
Pro	290	28	21.5	39.4	28.7	3.20	1.39	0.81	1.53	0.77	2.10
Total AA	291	24	399.0	669.2	487.7	44.66	10.90	0.94	12.50	0.92	3.57
DM	201	16	89.5	93.7	90.6	0.61	0.23	0.86	0.29	0.79	2.08
CP	276	31	425.7	639.1	483.7	38.75	4.82	0.99	5.55	0.98	6.99
CFat	197	14	21.8	41.9	31.2	4.22	2.98	0.50	3.27	0.40	1.29
CFibre	197	15	12.8	49.7	28.3	9.44	2.48	0.93	2.89	0.91	3.26
NDF[13]	207	2	56.4	324.0	188.2	54.04	36.98	0.53	41.35	0.42	1.31
ADF[14]	191	15	30.9	124.6	65.7	18.54	6.47	0.88	8.17	0.81	2.27
Lignin	40	4	0.1	0.3	0.1	0.06	0.06	0.15	0.07	-0.04	0.92
Ca	200	13	2.3	5.0	3.2	0.66	0.22	0.89	0.27	0.84	2.49
P	198	13	5.2	8.6	7.3	0.64	0.19	0.91	0.26	0.85	2.50

[1]O: number of outliers removed; [2]SD: Standard deviation; [3]SEC: Standard error of calibration; [4]RSQ: The fraction of the explained variance in the calibrations; [5]SECV: Standard error of cross validation; [6]1-VER: Fraction of explained variance in the cross validation; [7]RPD: Ratio of prediction to deviation (SD/SECV); [8]ADI tLys: Apparent ileal digestible total lysine; [9]AID rLys: Apparent ileal digestible reactive lysine; [10]SID tLys: Standardised ileal digestible total lysine; [11]SID rLys: Standardised ileal digestible reactive lysine; [12]TID tLys: True ileal digestible total lysine; [13]TID rLys: True ileal digestible reactive lysine; [14]NDF: Neutral detergent fibre; [15]ADF: Acid detergent fibre.