final report

Project code: A.MQT.0051

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Date published: July 2015

PUBLISHED BY
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Locked Bag 991
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Accreditation of VISNIR spectroscopy to support MSA grading of sheep meat

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government to support the research and development detailed in this publication.

Abstract

Initial economic modelling indicated that VISNIR spectroscopy could provide Australian lamb meat processors with a positive value proposition to cost effectively improve the consistency of grading of Meat Standards Australia (MSA) lamb. Previous MLA funded research (S.NGP.016D, P.RTC.031 and A.MQT.0051 (Phase 1)) identified the ability of VISNIR spectroscopy to objectively measure selected sheep meat quality traits including ultimate pH (pHu), ossification (lamb/hogget and mutton), intramuscular fat (IMF) content and meat colour in either hot or cold sheep meat carcasses (i.e. 30 mins and 24 hours post-slaughter respectively). However, further work was required to refine the accuracies of this objective carcass measurement technology to support a MSA grading system of sheep meat carcasses.

Work described in this final report further substantiates the effectiveness of VISNIR spectroscopy to predict ultimate pH, glycogen content, fresh meat colour and intramuscular fat content with accuracies approaching that required to attain highly reliable MSA grading standards. It also determined whether a VISNIR prediction model can be utilised to determine meat tenderness (i.e. shear force at day 5 of ageing). VISNIR prediction models for each of these meat quality traits, the impact of each of these traits on consumer sensory scores and the accuracies required for MSA accreditation are reported. Should these accuracy targets meet MSA accreditation standards, VISNIR grading of lamb will provide a substantial economic benefit to Industry.

Executive Summary

Previous MLA research (SNGP.016.D, PRTC.031 and Phase 1 of A.MQT.0051 projects) investigated the ability of VISNIR spectroscopy (using visible and near infrared wavelengths) to objectively measure selected sheep meat quality traits including ultimate pH, ossification, intramuscular fat (IMF) percentage and meat colour to support MSA grading of lamb either as hot or cold carcasses. Initial results supported the use of VISNIR spectroscopy to measure some of these traits with measurement accuracies approaching that required to attain highly reliable grading standards.

This project reports on the Phase 2 of A.MQT.0051 study commissioned by MLA to further refine the VISNIR meat quality prediction models important to lamb eating quality with the objective of achieving the grading accuracies required for the technology to achieve Aus Meat accreditation.

VISNIR validation models for predicting muscle glycogen content 30 minutes post slaughter, ultimate pH (pHu), intramuscular fat percentage and meat tenderness (shear force at day 5 of ageing) in LL muscle from VISNIR spectra acquired 24 hours post slaughter with 83%, 94%, 88% and 98% accuracy. The lamb carcasses were classified at being above or below nominated cut off values of total muscle glycogen content - 40 μmol/g, ultimate pH 5.70, IMF percentage - 3.5% and SF5 - 50 N. Similiar classification accuracies are reported for SM muscle. The VISNIR fresh meat colour calibration model was 78% for a nominated Hunter Lab L*-value of 37.8. Further analysis of the fresh meat colour data is required. These results are encouraging for Industry. Therefore, it is recommended that the project progress to a further validation phase to retest the reliability and accuracy of the VISNIR models across a wider cross-section of commercial sheep meat plants.

However, before VISNIR technology could be adopted by commercial industry there needs to be a major upgrade to the instrumentation and supporting software to industrialise the technology and make it much more operator friendly.

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1 Background

In recent years, Meat Standards Australia (MSA) has advanced the red meat industry's understanding and adoption of pre- and post-slaughter interventions to optimise eating quality. However, despite having stated a preference for objective carcass measurement technologies that are cost effective, robust and accurate to determine meat eating quality; industry is still largely reliant on a mix of objective and subjective carcass measures when grading lamb carcasses. Over the past decade, despite the best effort of the research community, no one technology has been identified with the ability to measure all commercially relevant quality traits that underpin MSA grading. Instead the most likely scenario for industry is the development and adoption of two or more objective carcass measurement technologies that are compatible with modern day industrial meat processing that can integrate seamlessly with MSA and commercial industry processing operations.

MLA has previously researched (SNGP.016D, PRTC.031 and A.MQT.0051) the ability of VISNIR spectroscopy (using visible and near infrared wavelengths) to objectively measure selected sheep meat quality traits including ultimate pH (pHu), ossification (lamb/hogget and mutton), intramuscular fat (IMF) content and meat colour in sheep meat to support MSA grading of sheep carcasses either as hot or cold carcasses. Initial results support the use of VISNIR spectroscopy to measure some of these traits with measurement accuracies approaching that required to attain highly reliable grading standards. It is reasonable to expect that, with further refinement to the methodology and expansion of the calibration and validation research data sets that underpin VISNIR prediction models the accuracies required for accreditation by MSA could be achieved.

Furthermore, economic modelling indicates that VISNIR technology provides lamb processors with a positive value proposition to cost effectively improve the consistency of grading of MSA lamb both within and across plants should these accuracy targets be achieved.

2 Projective Objectives

A VISNIR predictive regression model for ultimate pH, meat colour, tenderness (shear force) and intramuscular fat percentage of lamb is developed for validation.

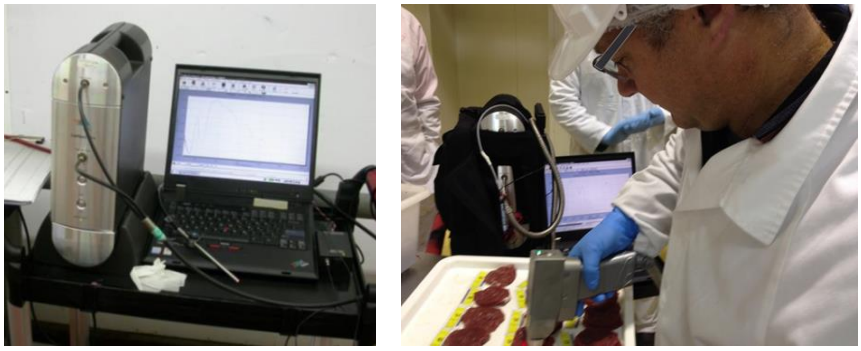
3 Materials and Methods

3.1 Lamb carcasses

Two hundred and five lamb carcasses were sourced from JBS Brooklyn over 7 kill days in mid-November, 2014. The lamb carcasses were selected on the following criteria: vendor identification, sex, carcass weight and fat score. The reason for selecting vendor identification was to ensure that lambs were sourced from a variety of regions across southern Australia. All carcasses were electrically stimulated post-dressing and trimmed according to AUS-MEAT specifications [1].

3.2 VISNIR Instrumentation

This study collected VISNIR spectra using 2 different VISNIR instrument configurations. The first VISNIR instrument configuration was fitted with the custom made pencil probe coupled with the ASD Terraspec 4 (Analytical Spectral Devices Inc., Boulder, Co., USA). The measuring field of view for the pencil probe is 0.12 cm^2. The second VISNIR instrument configuration was fitted with the custom made PAS handheld probe coupled with the ASD Labspec Pro (Analytical Spectral Devices Inc., Boulder, Co., USA). The measuring field of view for the PAS handheld probe is 0.26 cm^2. Figure 1 shows the 2 different VISNIR instrument configurations used in the study.



(a) (b)

Figure 1: VISNIR instrument configurations - (a) Pencil probe coupled with the ASD Terraspec 4 and (b) Handheld probe coupled with the ASD Labspec Pro.

3.3 VISNIR spectral measurements

VISNIR spectral measurements were collected in reflectance mode on two related muscle groups with regard to fibre type – the loin muscle (*M. longissimusthoracis et lumborum* - LL*)* and the topside muscle *(M. semimembranosus* - SM*)*. VISNIR spectra were collected 30 minutes and 24 hours post-slaughter using a Pencil probe instrument configuration and 24 hours post-slaughter using the Handheld probe instrument configuration.

3.3.1 VISNIR scanning protocol

LL muscle:

Thirty minutes post slaughter, 4 different VISNIR scanning locations were selected. At each location the Pencil probe was repositioned twice (see Figure 2A) to obtain 2 VISNIR spectra for this location. The exact position of the VISNIR scanning sites were as follows: scans 1-2 were between the 12/13 rib medial; scans 3-4 were between the 12/13rib lateral; scans 5-6 were between the 11/12 rib medial and scans 7-8 were between the 10/11 rib medial.

At 24 hours post slaughter using the Pencil probe instrument configuration, 2 different VISNIR scanning locations were selected and 2 VISNIR spectra was obtained at each location. The exact position of the VISNIR scanning sites were: scans 1-2 were between the 12/13 rib medial and scans 3-4 were between 11/12 rib lateral. These measurements were collected on the opposite side of the carcass to the 30 mins post-slaughter VISNIR measurements. The Pencil probe scanning locations were different to the 30 mins post-slaughter measurements due to the amount of time available to record VISNIR spectral measurements and additional measurements 24 hours post-slaughter.

For the Handheld probe instrument configuration, 2 different VISNIR scanning locations were selected and 1 VISNIR spectra was obtained at each location. This was performed on LL muscle in the carcass (LL in carcass – these measurements were taken where the carcass was broken at the 12 rib) and also LL muscle out of the carcass (LL out of carcass). The LL muscle sample out of the carcass was also used to obtain Videometer lab colour measurements. This VISNIR spectra acquisition was performed on bloomed muscle samples.

SM muscle:

Thirty minutes post slaughter, VISNIR spectra were obtained at 2 different scanning locations (see Figure 2B). At each location the custom made pencil probe was repositioned twice to obtain 2 VISNIR spectra for this location.

At 24 hours post-slaughter using the Handheld probe instrument configuration, 2 different VISNIR scanning locations were selected and 1 VISNIR spectra was obtained at each location. This was performed on SM muscle out of the carcass and this sample was also used to obtain Videometer Lab colour measurements. This spectra acquisition was performed on bloomed muscle samples.

Both VISNIR instrument configurations were set to collect 15 scans per spectra acquisitition and the orientation of the custom made pencil probe was always perpendicular to muscle fibres. The Handheld probe was always re-positioned for each spectra acquisitition. It took less than 1 second to obtain and record each VISNIR spectra. A summary of the VISNIR scanning protocol is shown in Table 2. Figure 2 depicts the location of VISNIR spectra acquisition 30 minutes post-slaughter on LL muscle through the mantle (A) and SM muscle (B) using the VISNIR pencil probe coupled with the ASD Terraspec 4.

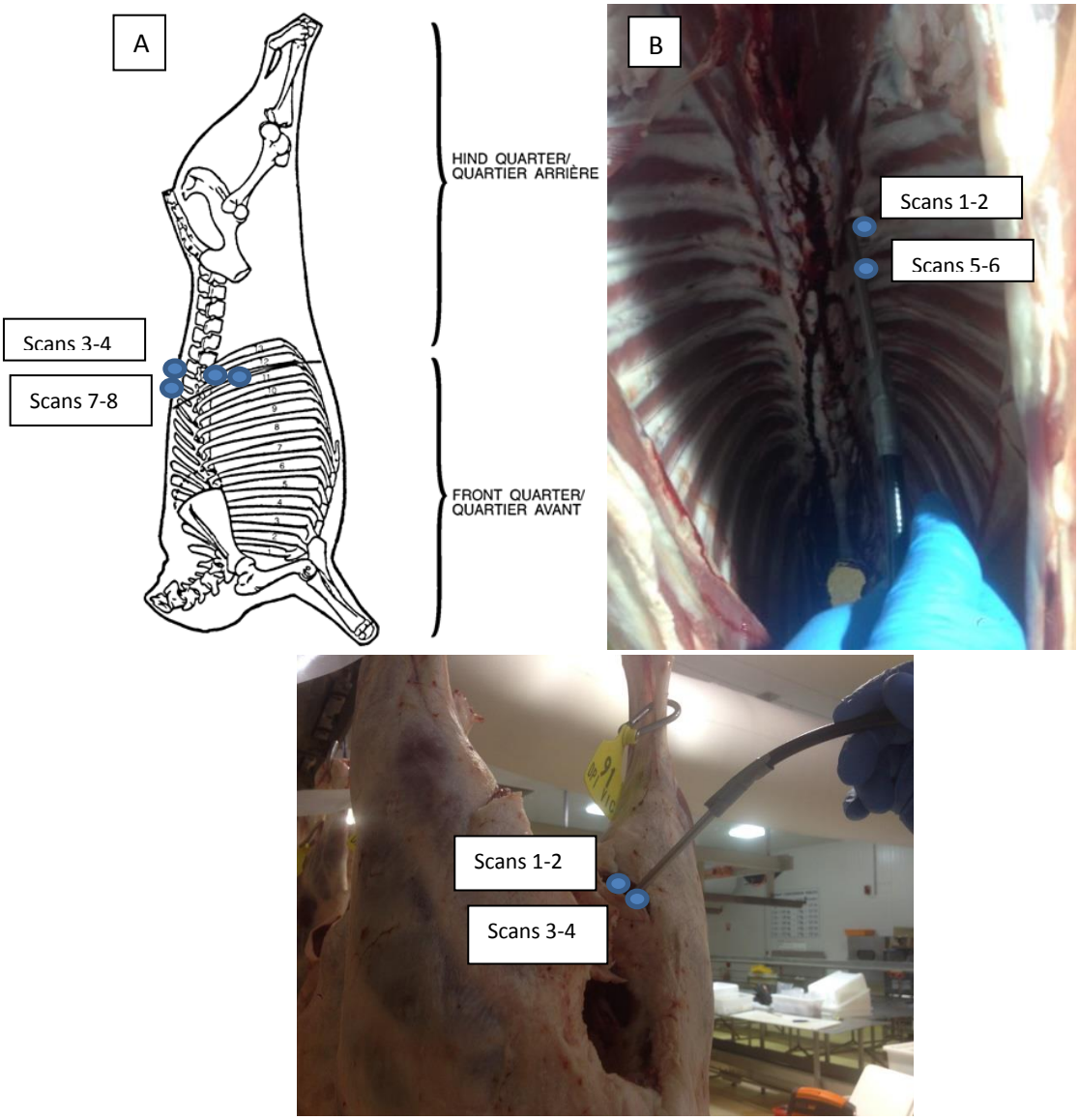


Figure 2: Images depicting VISNIR spectra acquisition 30 minutes post-slaughter on LL muscle (A) and SM muscle (B) using the Pencil probe coupled with the ASD Terraspec 4.

3.4 Carcass sampling and measurement protocol

The origin of the lamb (i.e. vendor identification), sex, hot carcass weight and fat score was recorded for each lamb carcass. Thirty minutes post slaughter, a 5 gram sample of both LL and SM was removed from each carcass and sliced into 1g pieces. All the fat was removed from each muscle sample. Duplicate 1g samples were then snap frozen in liquid nitrogen and stored at -80^{o}C for future glycogen and lactate biochemical analysis (Section 3.4.4, Figure 3A). Using a GR knife, tissue depth over the 12th rib was recorded, 110mm from the midline of the carcass [2]. The pH and temperature of both muscles was then recorded by 3 independent assessors using the following pH meter (Model: WP-80, Manufacturer: TPS Pty Ltd, Springwood, Qld, Australia) fitted with a temperature probe [3]. The pH meter was calibrated every day using buffers of pH 4.0 and pH 7.0 at chiller temperature (2^{o}C).

Approximately 24 hours post-slaughter, the ultimate pH and temperature of the LL and SM muscle was recorded by 3 independent assessors according to McPhail, *et. al.* [3]. Each carcass was then cut at the 12th rib through the LL muscle and the cut surface was exposed to air for 30 – 40 min prior to fresh meat colour measurement (Section 3.4.1).

Table 1: Summary of VISNIR spectra scans collected on each carcass.

Time	Instrument	Muscle	Locations	Scans per location	Total number of scans
30 mins post-slaughter	Pencil probe	LL	4	2	8
		SM	2	2	4
24 hours post-slaughter	Pencil probe	LL (no bloom)	2	2	4
		SM (no bloom)	1	2	2
	Handheld probe (with 30 mins bloom time)	LL in carcass	2	1	2
		LL out of carcass	2	1	2
		SM	2	1	2
Total VISNIR spectra carcass					24 (16 LL and 8 SM)

Approximately 300g (i.e. 200 mm) of the eye of the shortloin (LL) was then removed from the left side of the carcass and all subcutaneous fat and epimysium (silver skin) was removed. From the head end, two 40g samples were collected for: (1) intramuscular fat, mineral and fatty acid content and (2) Videometer Lab (Manufacturer: Videometer A/S, Hørsholm, Denmark) colour measurement. The intramuscular fat, mineral and fatty acid content sample was frozen at -20^{o}C on day 1 post-slaughter. The Videometer lab colour measurement samples was butterflied and placed on a tray to allow to bloom for at least 30 minutes prior to measurement. Two 65g samples were then taken from the LL for shear force day 5 (SF5) and compression analysis (if required). Each sample was vacuum packed and stored at 2^{o}C for another 4 days prior to storage at -20^{o}C. One 40g sample was then collected on a subset of samples for driploss analysis and followed by another 40g sample for Vitamin E analysis (if required). The remainder of the tail end of the LL samples was vacuum packed and stored at -20^{o}C.

The topside (SM) was then removed from the left hindleg (Figure 3B). Once removed the cap was separated from the SM muscle, a 40g slice of the SM muscle was removed from the narrow end for Videometer lab (Videometer A/S, Hørsholm, Denmark) colour measurement. This sample was placed on a tray to allow to bloom for at least 30 minutes prior to measurement. Two 65g samples were then taken from the SM for shear force day 5 (SF5) and compression analysis (if required). Each sample was vacuum packed and stored at 2^{o}C for another 4 days prior to storage at -20^{o}C. Finally, one 40g sample was collected for intramuscular fat, mineral and fatty acid content. This sample was stored at -20^{o}C on day 1 post-slaughter. A schematic diagram depicting the LL and SM muscle sampling protocol is shown in Figure 4.

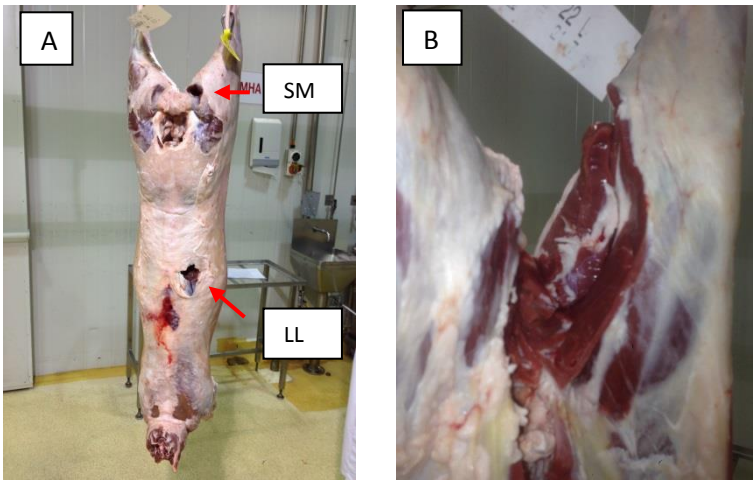


Figure 3: Carcass images depicting the location were individual muscle samples were extracted from the carcass. (A) Loin (LL) and topside (SM) muscle extracted from carcass 30 mins post-slaughter and (B) Carcass depicting the topside muscle extracted, 24 hours post-slaughter.

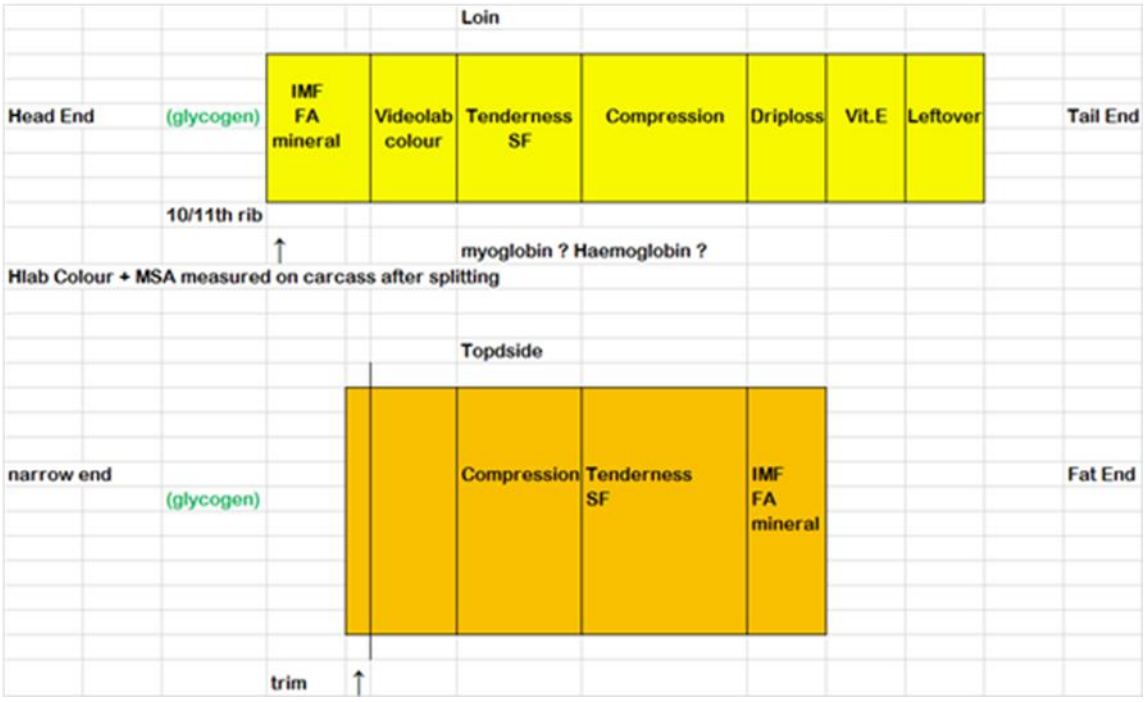


Figure 4: Schematic diagram depicting the LL and SM muscle sampling protocol used in this study.

3.4.1 Fresh meat colour

Fresh meat colour was measured 24 hours post slaughter using three different colour instruments: (a) visual colour measurement using MSA meat colour chips and marbling score (b) Videometer lab, (c) HunterLab colour meter and (d) Minolta Chromameter.

(a) Visual meat colour and marbling assessment using MSA accredited graders

Visual meat colour was performed by MSA accredited graders in accordance with MSA guidelines for beef – Meat Colour in the AUS-MEAT National Accreditation Standards – Standards for Chiller Assessment. MSA marbling was assessed using MSA marbling standard photographs for beef in accordance with AUS-MEAT guidelines. Each cut was given a grading score in the range of 100 to 1190 in increments of 10. Visual meat colour and marbling assessments were performed on LL and SM muscle samples used for Videometer Lab meat colour assessment.

(b) Videometer Lab:

Videometer Lab measurements were performed on a 30mm cut slice of LL and SM muscle. The cut muscle was placed on a plastic tray and allowed to bloom for at least 30 minutes at 8^{o}C prior to measurement. Videometer lab measurements were performed according to the manufacturer's instructions (Videometer A/S, Hørsholm, Denmark). Figure 5 is picture of the Videometer Lab.

(c) HunterLab colour meter:

Fresh meat colour measurements using the HunterLab colour instrument were performed on a 30mm cut slice of LL (loin) and SM (topside). The cut muscle was placed on a plastic tray and allowed to bloom for at least 30 minutes at 8^{o}C prior to measurement. HunterLab meat

colour measurements were performed using a HunterLab instrument (HunterLab Miniscan, TM XEPlus 45/10, Reston, VA, USA) with the light source set at D65/10 [4].

(d) Minolta chromameter:
Fresh meat colour measurements using the Minolta chromameter, D65 iluminant with a 2^{o} standard observer and 8mm aperture were performed according to Warner, *et. al.* [5]. Prior to the collection of fresh meat colour measurements, the chromameter was calibrated on a white tile before measurement. Once calibrated, the *L** (lightness), *a** (redness) and *b** (yellowness) were measured on each LL muscle in carcass. This was performed after a blooming period of 30 – 40 minutes. Minolta chromameter fresh meat colour measurements were also performed on the SM muscle out of carcass. Once again, the SM samples were allowed bloom for a period of 30 – 40 mins prior to measurement. All fresh meat colour measurements were recorded in triplicate and the mean value was used for analysis.

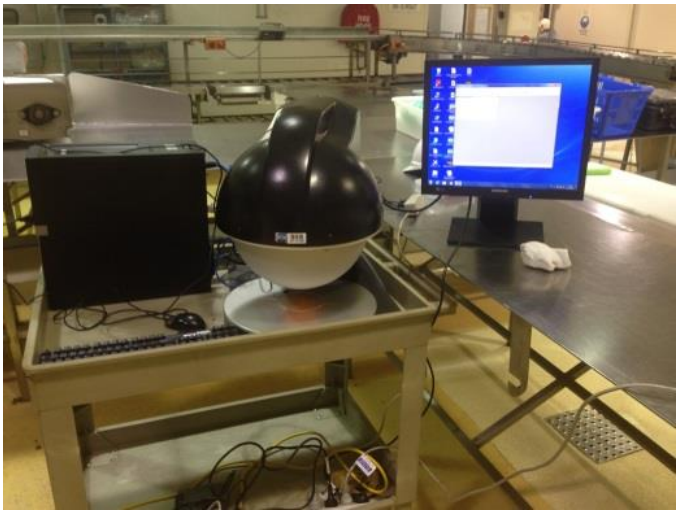


Figure 5: The Videometer Lab used to measure meat colour and intramuscular fat content of lamb (Videometer A/S, Hørsholm, Denmark).

3.4.2 Shear force at day 5 of ageing

Shear force analysis at day 5 of ageing for LL and SM muscle samples was performed by NSW DPI according to the published method of Hopkins and Thompson [6]. Muscle samples were taken directly from the freezer and placed into a pre-heated waterbath at 71^{o}C and cooked for 35 mins. After cooking, all samples were cooled under running water for 30 mins. The next day after cooking, six samples approximately 30 – 40 mm long and 10 mm across were cut from each LL and SM sample ensuring that the muscle fibres ran along the long axis of the sample and that any fat or connective tissue was avoided. Shear force measurements were determined using a texture analyser (Model: LRX, Manufacturer: Lloyd Instruments, Hampshire, UK) with a set cross head space at 200mm/min and a fitted with a 1 kN load cell. The samples were sheared with the blade in an inverted V-blade positioned perpendicular to muscle fibre orientation.

3.4.3 Intramuscular fat

The intramuscular fat content of LL and SM muscle samples were determined using a modified version of AOAC Method 960.39 (AOAC, 2007) for ether-extractable fat. Approximately 40 g of diced wet muscle was collected in 50 ml Falcon tubes and stored at – 20°C until subsequent freeze drying. Prior to freeze drying, the wet muscle weight of the sample was recorded. Each sample was then completely freeze dried and the dry muscle weight of the sample was then recorded. After freezer drying the sample was ground using a FOSS Knifetech™ 1095 sample mill (FOSS Pacific, North Ryde, NSW). The IMF content of each sample was then determined using the soxhlet IMF extraction method [7].

3.4.4 Muscle glycogen and lactate content

Muscle glycogen and lactate content was determined on LL and SM muscle collected 30 minutes post-slaughter performed according to Coombes, *et. al.*, [8]. All assays were performed in duplicate and repeated if the measurement had a error greater that 10% between the duplicates. Total muscle glycogen content was the sum of glycogen content and halving the lactate content [8]. The total glycogen content was expressed as μmol/g of wet tissue and reflects the muscle glycogen content at the time of slaughter. Like Coombes, *et. al.*, [8], the authors note that lactate may have been in the muscle prior to slaughter and acknowledge that this lactate will contribute to the ultimate pH of the muscle. Hence, this is why the lactate concentration was included in the calculation of total glycogen content of each muscle sample.

3.5 Statistical analysis

Preprocessing of VIS-NIR spectra:

VISNIR spectra were acquired with the Pencil probe coupled with the ASD Terraspec 4 from LL and SM muscles from each carcass 30 minutes post mortem (i.e. hot carcasses). Prior to attempting to create a PLS regression model capable of predicting how meat colour has developed in carcasses 24 hours post-slaughter, the VISNIR spectra were preprocessed. This was done in two steps: (1) the VISNIR spectra are smoothed using a Gaussian filter with gap width of 11nm in the entire range from 350nm to 2500nm and (2) the smoothed VISNIR spectra were subsequently subjected to a second derivative transformation using a gap of 5nm. This choice of signal preprocessing will have an effect on the outcome of the modeling. Selecting a gap size of 5nm removed as much noise as possible in the VISNIR spectra without blurring the underlying signal.

Modelling strategy:

Visual inspection of the preprocessed VISNIR spectra showed obvious noise at wavelength regions below 400nm and above 1750nm. This noise was attributed to attenuation in the fibre optics of the probe. PLS regression models were then developed using the preprocessed spectra from 400nm to 1000nm as the input data (x-data).

The wavelength region between 400nm to 700nm is visible to the naked eye. Therefore, it would be advantageous to restrict initial VISNIR analysis to this region. It should also be noted that when VISNIR measurements were collected using the pencil probe, the operator had no knowledge of where the probe tip was placed in the muscle or what structures are actually being measured. Thus, it is likely that the short wave part of the VISNIR spectrum may contribute to assisting the efficacy of the PLS regression model by compensating for spectral

interference from collagen and fat tissue that in many cases will dominate over meat in the acquired spectra. The y-data could be either of the L*-values from the three instruments.

4 Results

4.1 Summary of carcass measurements data

Summary tables of all carcass measurements and muscle sample collection are listed below. Table 2 groups the carcass weight, GR fat depth and sex data of all animals used in this study. These sheep were sourced from 25 different vendors from across southern Australia and 4 different market lots from Shepparton, Corowa, Bendigo and Ballarat. These data were collected over 7 different kill days in mid-November, 2014.

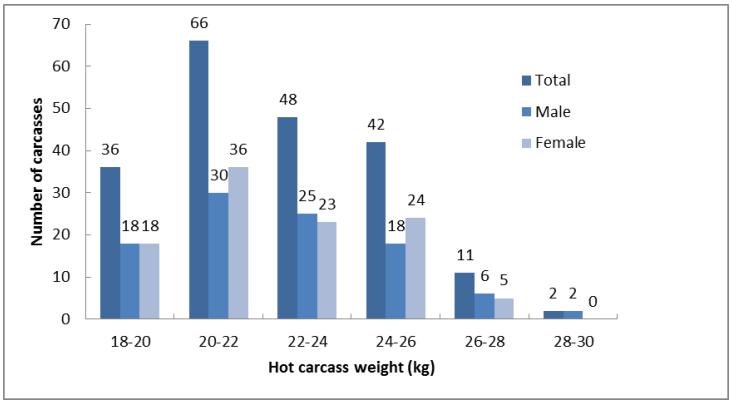
Table 2: Hot carcass weight, sex and GR fat depth distribution of all 205 randomly selected lamb carcasses killed over a period of 7 kill days in mid-November 2014.

HCWT (kg)	# Carcasses	Sex		GR fat depth distribution			
		Castrate Male	Female	<5mm	<10mm	<15mm	<20mm
18-20	36	18	18	8	24	4	0
20-22	66	30	36	14	41	11	0
22-24	48	25	23	1	17	28	2
24-26	42	18	24	0	14	26	2
26-28	11	6	5	0	2	5	4
28-30	2	2	0	0	0	1	1
Total	205	99	106	23	98	75	9

Table 3: Descriptive unadjusted statistics for hot carcass weight and meat quality traits.

Trait	Mean	Std Dev	Maximum	Minimum	Range
HCWT (kg)	22.35	2.39	29.30	18.10	11.2
GR fat depth (mm)	8.95	3.15	17.00	3.00	14
LL muscle					
Ultimate pH	5.63	0.13	6.76	5.41	1.35
SF5 (N)	34.16	9.29	73.42	19.55	53.87
IMF content (%)	3.30	0.99	6.47	1.22	5.25
Glycogen content (μmol/g)	61.71	12.45	95.09	19.08	76.01
SM muscle					
Ultimate pH	5.64	0.14	6.86	5.40	1.46
SF5 (N)	51.54	10.63	76.25	23.01	53.24
IMF content (%)	3.15	0.86	5.59	1.37	4.22
Glycogen content (μmol/g)	60.52	11.14	88.03	15.59	72.44

(A)



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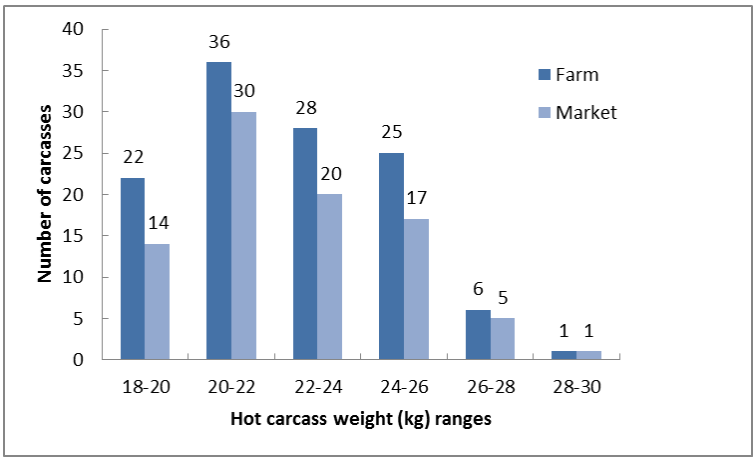

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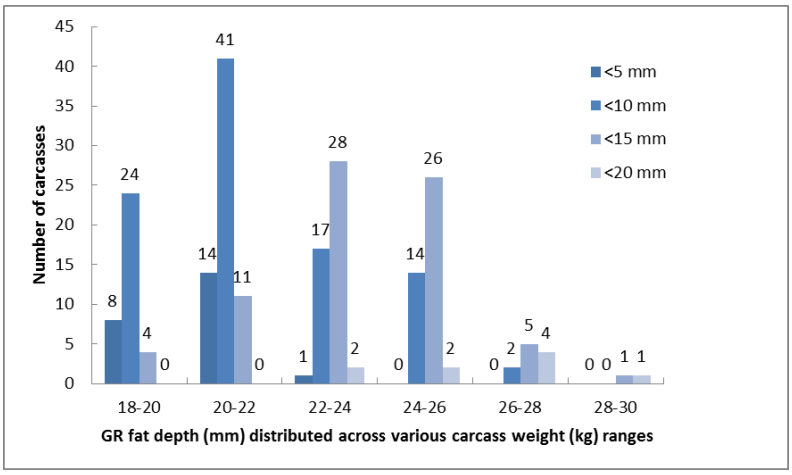


Figure 6: Distribution of hot carcass weight and sex (A), source vendor lot (farm or market) (B) and GR fat depth related to hot carcass weight catergories (C) from all 205 lamb carcasses sampled. These lambs were slaughtered over 7 kill days in mid-November 2014 and sourced from 25 different vendor lots and from 4 different markets.

4.2 Ultimate pH

For ultimate pH, a pH value less than 5.7 was considered to be an acceptable target for lamb to ensure consumer sensory satisfaction and acceptable eating quality. Figure 7 shows the distribution of ultimate pH for both LL and SM muscle sampled in this study.

It should be noted that where possible, the ultimate pH value represented Figure 7 is the average ultimate pH measurement from 3 independent assessors. The main reason for using the average of 3 independent ultimate pH readings for developing the VISNIR regression model was to increase the accuracy of ultimate pH reading for each sample. This should increase the accuracy of the ultimate pH reading to greater than ±0.2. The VISNIR spectra used to generate this model were generated using the Handheld probe coupled with the ASD Labspec, 24 hours post-slaughter.

Table 4 and Table 5 represents the statistical interpretation and the success of VISNIR predicting ultimate pH reference measurement for LL in and out of carcass and SM muscle, respectively. Figure 8 highlights a scatter plot of VISNIR predicted ultimate pH versus the reference ultimate pH values for LL muscle in carcass.

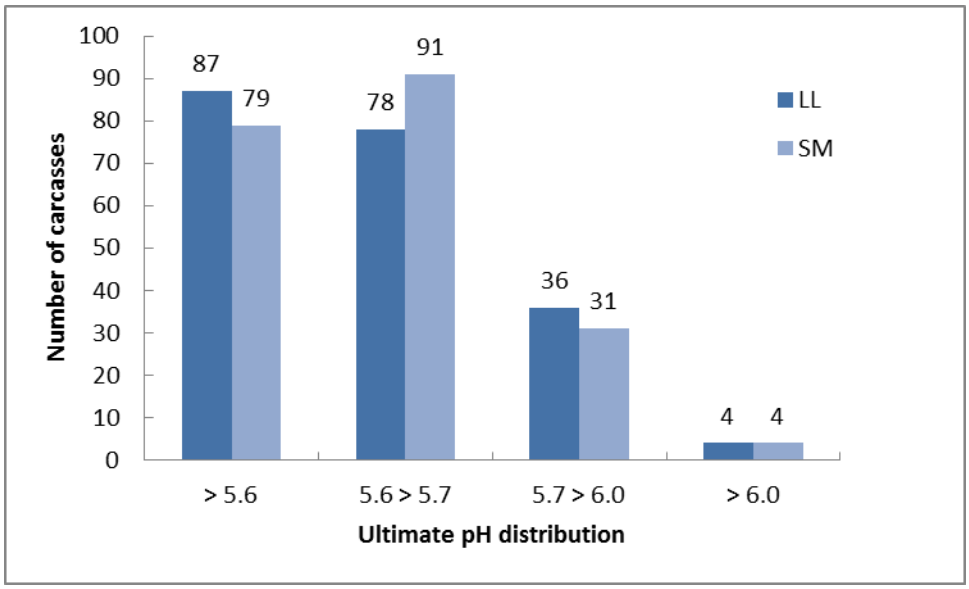


Figure 7: Distribution of ultimate pH data recorded on loin (LL) and topside (SM) muscle from all 205 lamb carcasses. This data is the averaged ultimate pH reading from 3 independent assessors.

Table 4: Ultimate pH VISNIR regression model statistical interpretation for loin (LL) in and out of carcass and topside (SM) muscle. This VISNIR regression model was generated using the custom made PAS handheld probe coupled with the ASD Labspec Pro, 24 hours post-slaughter.

Ultimate pH	LL (in Carcass)	LL (out Carcass)	SM
SECV[1]	0.1	0.1	0.1
Uncertainty[2]	±0.2	±0.2	±0.2
R-squared[3]	0.49	0.37	0.70
Slope[4]	1.23	1.12	1.10
Offset[5]	-1.30	-0.68	-0.59
BIAS[6]	-0.07	-0.05	0.03

[1]SECV – standard error of cross validation. The standard deviation of the differences between the predicted and measured values.

[2]Uncertainty – degree of uncertainty associated with the precision of the measurement.

[3]R-squared – multiple correlation coefficient of the line of best fit obtain when plotting the predicted values against the measured values.

[4]Slope – gradient of the line of best fit obtain when plotting the predicted values against the measured values.

[5]Offset – intercept of the line of best fit obtain when plotting the predicted values against the measured values.

[6]Bias - mean value over all points that either lie systematically above (or below) the regression line. A value close to zero indicates a random distribution of points about the regression line.

Table 5: Success of VISNIR regression model predicting ultimate pH of LL and SM muscle from lamb carcasses sampled in this study.

Ultimate pH	LL (in Carcass)	LL (out Carcass)	SM
Cutoff	5.7	5.7	5.7
Correctly Assigned	169	183	192
False Pass	5	9	6
False Fail	6	11	7
%Correctly Assigned	94%	90%	94%
%False Pass	3%	4%	3%
%False Fail	3%	5%	3%

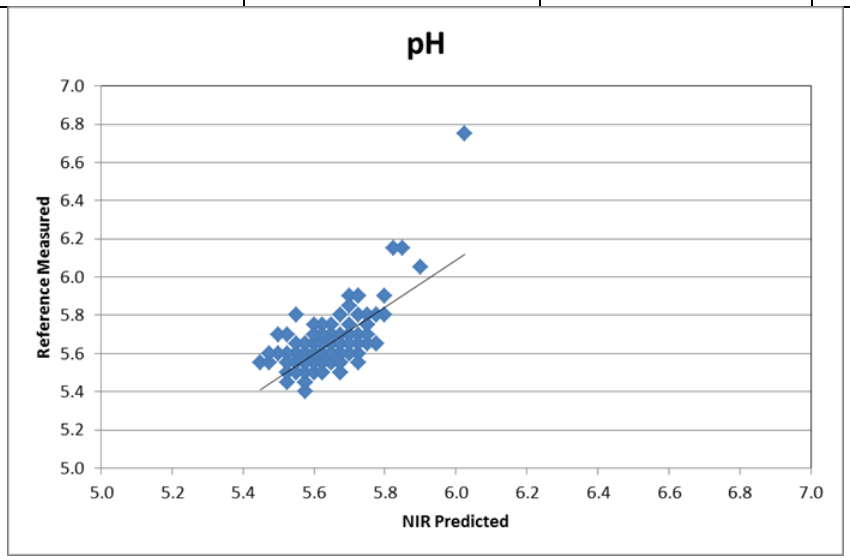


Figure 8: Scatter plot representing VISNIR predicted ultimate pH versus the reference ultimate pH value for loin (LL) in carcass measurements.

4.3 Glycogen content

The glycogen and lactate content of LL and SM muscles was determined in all 205 carcasses. The total glycogen content was then determined by summing the glycogen content and half the lactate concentration. This adjusts for any glycolysis that occurs post-slaughter. Figure 9 shows the relationship between ultimate pH and muscle glycogen content for LL and SM muscle. This figure showed that ultimate pH was not impacted on by where the lambs were sourced (i.e. direct vendor lots or saleyards). However, the source of lambs were not tested in the development of the VISNIR regression model used to predict total glycogen content. This was because the main aim of this work was to develop VISNIR regression models suitable for all sources of lambs killed in the abattoir.

(A) (B)

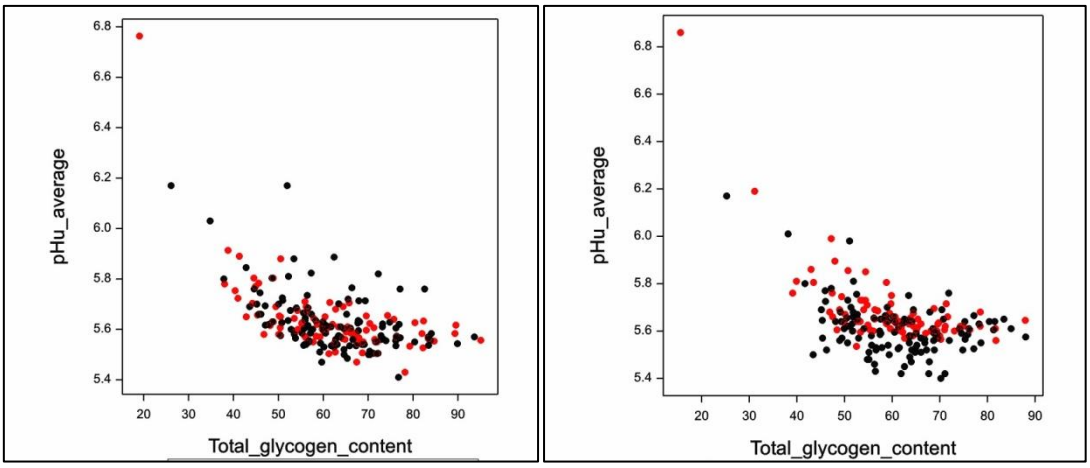


Figure 9: Scatter plot representing the total glycogen content (µmol/g) versus ultimate pH of (A) LL and (B) SM muscles of all 205 lambs sampled in this study. Red dots represents lambs sourced from the saleyards and black dots represents lambs sourced from direct vendor lots.

Data modelling to predict ultimate pH in the LL muscle:

Various models for predicting the ultimate pH using total muscle glycogen content in the LL muscle determined 30 minutes post-slaughter are presented below. All models examined the additional effects of sex, kill day and plate run (i.e. distinct measurement grouping of samples per glycogen measurement batch). Sex did not have any significant effect on the models developed and plate run was fitted as a random term. The GenStat linear coefficienct regression model developed to predict ultimate pH of carcasses using the muscle's total glycogen content in LL muscle is shown below; along with the coefficients for the model (see Table 6).

Table 6: Linear coefficient regression model that predicts ultimate pH in LL using muscle total glycogen content.

Coefficients	LL muscle Model 1 (n = 202)
Constant	5.678 ± 0.0203
Total glycogen	-0.0253 ± 0.003558

Linear coefficient of regression analysis to predict ultimate pH in for LL muscle using total glycogen content

Response variate: pHu_av
Fixed model: Constant + Total_Gly + Total_Gly2 + Day
Random model: Plate_run
Number of units: 202

Tests for fixed effects

Sequentially adding terms to fixed model

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Total_Gly	74.87	1	74.87	190.5	<0.001
Total_Gly2	37.38	1	37.38	189.9	<0.001
Day	20.68	5	4.00	28.7	0.007

Dropping individual terms from full fixed model

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Total_Gly	50.48	1	50.48	190.5	<0.001
Total_Gly2	37.15	1	37.15	189.9	<0.001
Day	20.68	5	4.00	28.7	0.007

Table of effects for Constant

5.678 Standard error: 0.0203

Table of effects for Total_Gly

-0.02528 Standard error: 0.003558

Table of effects for Total_Gly2

0.0001688 Standard error: 0.00002769

Table of effects for Day

Day	1	2	3	4	5
	0.00000	-0.07750	-0.05886	-0.07495	-0.07932

Day	6

-0.03482

Standard errors of differences

Average:	0.02383
Maximum:	0.02803
Minimum:	0.01822

Average variance of differences: 0.0005771

Table of predicted means for Constant

5.624 Standard error: 0.0110

Table of predicted means for Day

Day	1	2	3	4	5	6
	5.678	5.601	5.620	5.603	5.599	5.644

Standard errors of differences

Average:	0.02383
Maximum:	0.02803
Minimum:	0.01822

Average variance of differences: 0.0005771

Predicting total glycogen content using VISNIR:

Table 7 reveals the statistical interpretation for the VISNIR regression model developed for total glycogen content for LL muscle in carcass and SM muscle using the PAS handheld probe instrument configuration, 24 hours post-slaughter. Individual VISNIR regression models were developed for each muscle. These models were then used to classify carcasses at 2 different total glycogen categories – 40 μmol/g or 50 μmol/g. Figure 10 represents the scatter plot for VISNIR predicted total glycogen content versus the reference total glycogen content measurment in LL muscle. Table 8 reveals the success of the LL and SM muscle VISNIR regression models at predicting total glycogen content of muscle. The VISNIR regression model developed for total glycogen content in muscle had 98% accuracy at predicting total glycogen content above 40 μmol/g in LL and SM muscle. However, the accuracy of this prediction decreases slightly when predicting total glycogen content above 50 μmol/g in LL and SM muscle to greater than 87%.

Table 7: Total glycogen content VISNIR regression model statistical interpretation for loin (LL) in carcass and topside (SM) muscle. This VISNIR regression model was generated using the custom made PAS handheld probe coupled with the ASD Labspec Pro, 24 hours post-slaughter.

Glycogen content	LL (in carcass)	SM
Terms	6	6
SECV	9.0	8.1
R-squared	0.51	0.48
Slope	1.20	1.20
Intercept	-12.10	-11.88
Bias	-5.20	-4.40

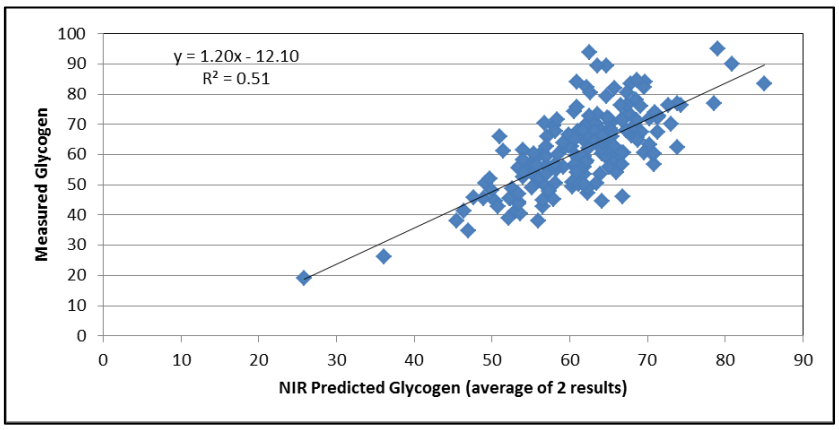


Figure 10: Scatter plot representing VISNIR predicted total glycogen content versus the reference measured total glycogen content for loin (LL) in carcass measurements.

Table 8: Success of VISNIR regression model at classifying carcasses into 2 different total glycogen content categories – either 40 μmol/g or 50 μmol/g.

Total glycogen content	LL in carcass		SM	
Cutoff (μmol/g)	40	50	40	50
Correctly Assigned	198	177	176	156
False Pass	0	2	0	2
False Fail	4	23	4	22
%Correct	98%	88%	98%	87%
%False Pass	0%	1%	0%	1%
%False Fail	2%	11%	2%	12%

4.4 Fresh meat colour

Prior to the development of VISNIR regression models for fresh meat colour, the relationship between Aus Meat beef colour grading chips and the 3 different colour reference measurement instruments used in this study were determined. Figure 11 shows the relationship between Aus Meat beef colour chip and L*-, a* and b*-value all 3 instruments. When comparing the dynamic range of the Hunter Lab against the Videometer Lab instrument, there is a close relationship between the a*- and b*-values of both instruments; however, the Videometer Lab has a greater dynamic range for L*-value when measuring the Aus Meat beef colour chips (Figure 11A). Nearly the opposite relationship is observed between the Minolta Chromameter and the Videometer Lab. The Videometer Lab has a greater dynamic range for both the a*- and b*-values; whereas the L*-value observed for both instruments have a similar dynamic range (Figure 11B).

(A) (B)

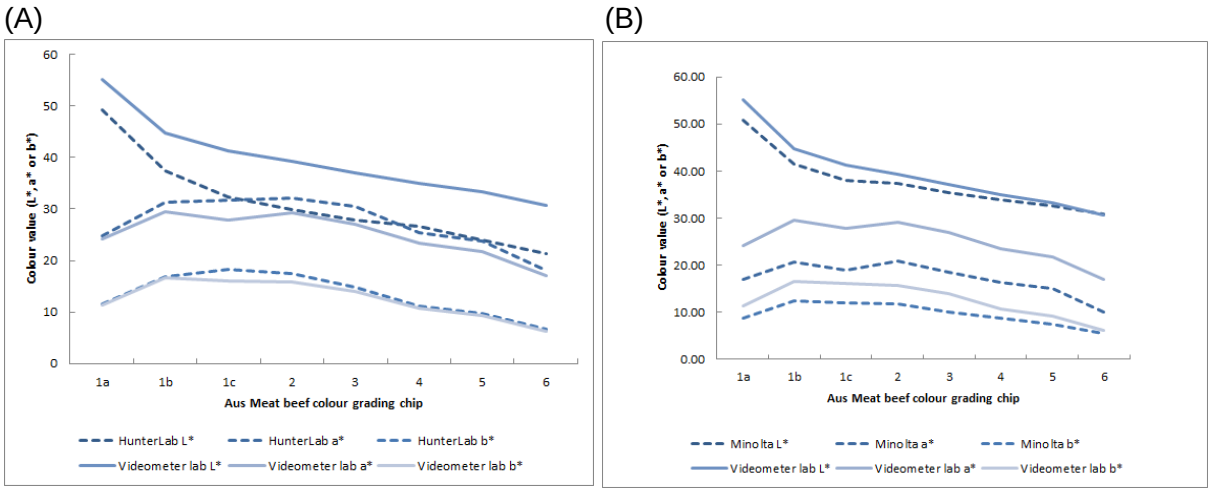


Figure 11: The relationship between the 3 colour reference measurement instruments for determining L*-, a*- and b*-value of Aus Meat beef colour grading chips. (A) HunterLab versus Videometer Lab and (B) Minolta chromameter versus Videometer Lab.

Visual colour assessment using Aus Meat beef colour grading chips:

All LL and SM muscle samples were independently assessed by 2 qualified MSA beef graders using AusMeat beef colour grading chips. The distribution of Aus Meat beef colour grading chip score for LL and SM muscles of all 205 lamb carcasses are shown in Figure 12.

Figure 13 plots the HunterLab instrument L*-value of LL muscles versus the average visual colour assessment provided by the two qualified MSA beef graders. It should be noted that carcass 187 was removed from this plot because this data point was an outlier.

The correlation between visual colour assessment and L*-values for all 3 reference colour instruments was R ≈ -0.4. However, it should be noted that if the two LL muscle samples that were given the visual colour assessment score of 7 were removed from the analysis, the correlations will almost be reduced to zero. Thesse data suggest there is little to no relationship between visual colour assessment using beef colour grading chips and all 3 reference colour instruments when assessing lamb.

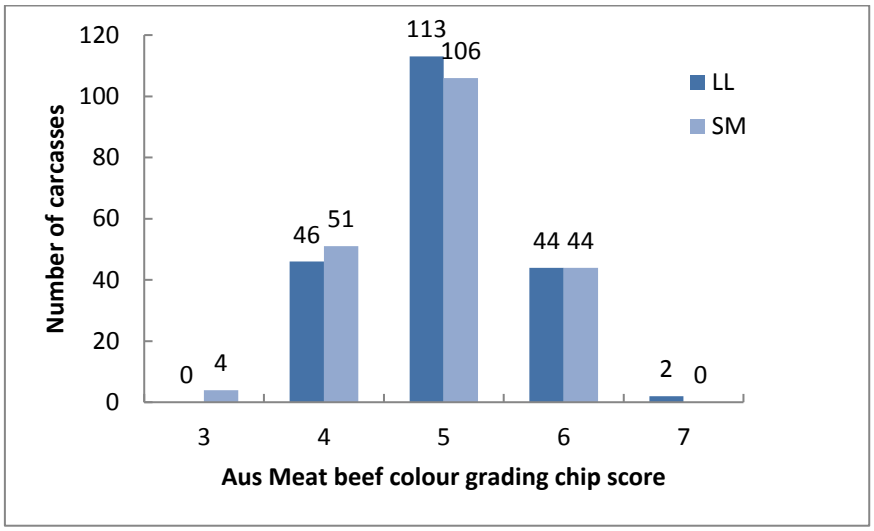


Figure 12: Distribution of Aus Meat beef colour grading chip score for LL and SM muscle of all 205 lamb carcasses.

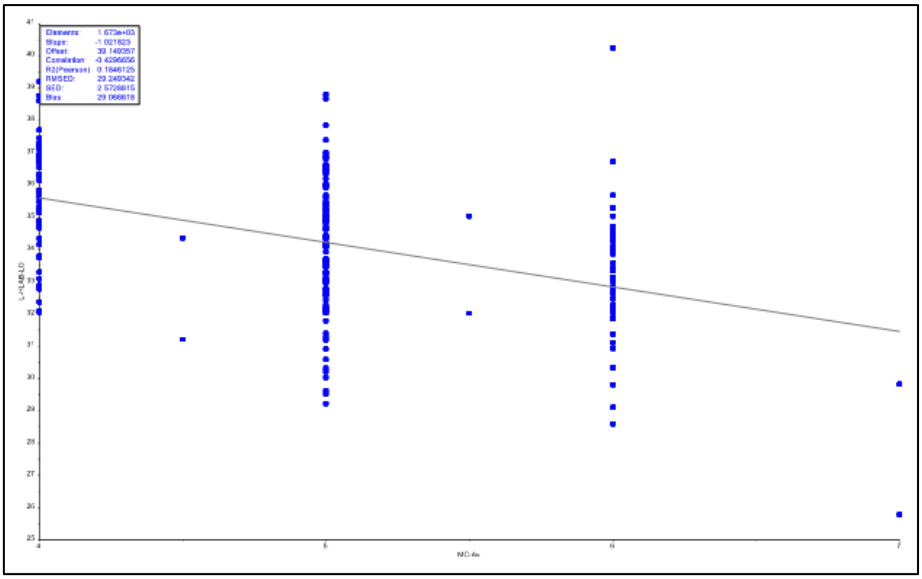


Figure 13: The relationship between visual colour assessment using qualified assessors and Aus Meat beef colour grading chips and HunterLab L*-values for 204 lamb carcasses.

Fresh meat colour assessment using 3 reference colour instruments:

Fresh meat colour measurements were collected on approximately 24 hours post-slaughter using 3 different reference colour measurement instruments (i.e. Minolta Chromometer, HunterLab colour instrument and Videometer Lab). Reference colour measurements were collected on bloomed LL and SM muscle in and out of carcass. The main difference between the 3 colour instruments was in the illumination of the sample; the area that is being measured; and how a representative measurement is obtained on a sample that is rather inhomogeneous.

The Minolta and HunterLab instruments illuminate the surface of the sample at a fixed angle whereas the Videometer Lab applies diffuse light. This difference resulted in the Videometer Lab judging the meat samples more dark than the other two colour measuring instruments. This result is an important finding considering several hyperstpectral devices are currently being developed for application in the meat processing sector to determine meat quality traits such as fresh meat colour. In regard to fresh meat colour, there will be a need to develop an indepth understanding of how the results obtained from each hyperspectral device (i.e. diffuse light) and how this compares to the traditional Minolta Chromameter or HunterLab instruments. The result obtain from the Videometer Lab was opposite the result we observed when assessing L*, a*- and b*-values of the Aus Meat beef colour grading chips.

Figure 14 shows 2 Videometer Lab images from 2 “butterflied” loin cuts - Carcass 166 and 179. The main reason for butterflied loin cuts was to increase the surface area scanned by the Videometer Lab to enable greater representation of IMF content and fresh meat colour. Figure 15 shows the correlation for all 205 LL muscle L*-value for bloomed fresh meat colour measurements for (A) HunterLab instrument versus Videometer Lab (B) Minolta chromometer versus Videometer Lab and (C) HunterLab instrument versus Minolta chromometer. For all 205 LL muscles, the correlation between L*-value measured by Videometer Lab and Minolta chromameter was 0.37 and Minotla chromameter and HunterLab instrument was 0.23.

The redness of the sample is typically reflected in the a*-value calculated by the colour measuring device. For all 205 loin muscles, the correlation between a*-values measured by the Minolta chromameter and HunterLab instrument was 0.54. Whilst, the correlation between Videometer Lab and HunterLab instrument was 0.45.

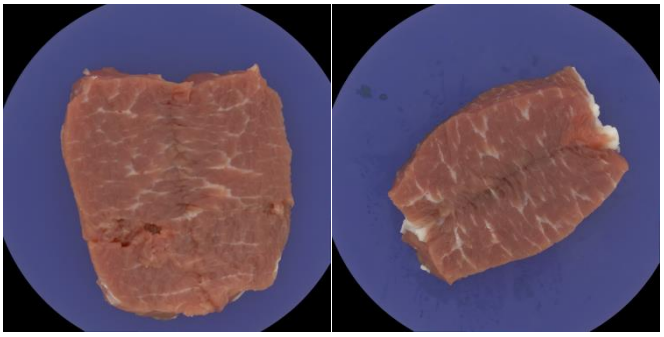
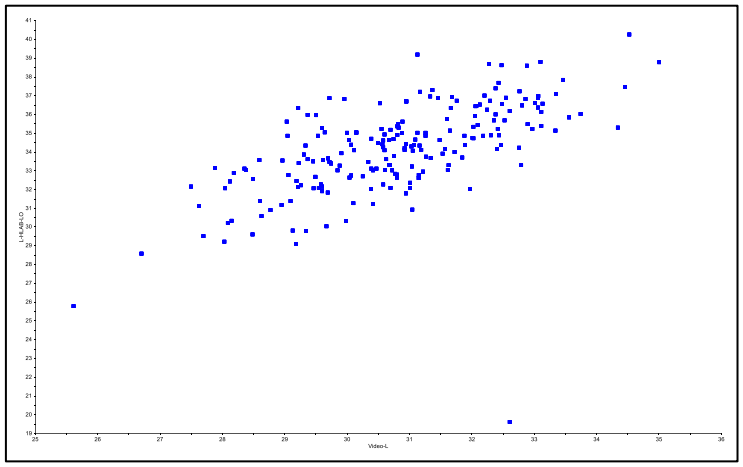
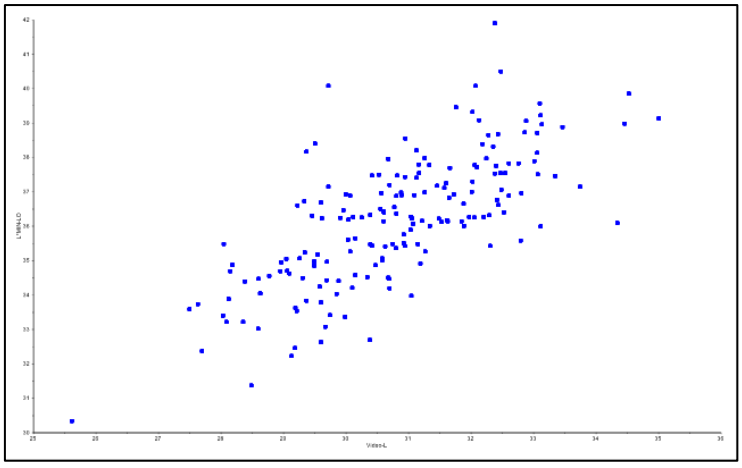


Figure 14: Videometer Lab colour measurements of butterflied LL muscles samples extracted from (A) carcass 166 - Minolta chromometer L*-value = 40.24 and a*-value = 15.72 and (B) carcass 179 – Minolta chromometer L*-value = 35.93 and a*-value = 16.83.

(A)



(B)



(C)

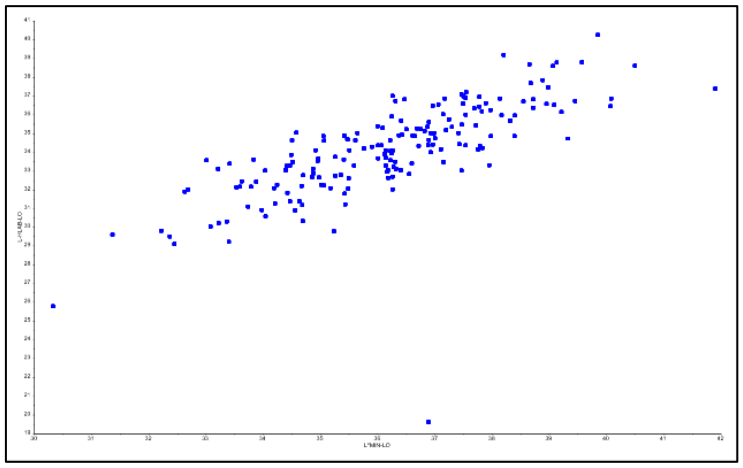


Figure 15: Correlation of LL muscle L*-value for bloomed fresh meat colour measurements for (A) HunterLab instrument versus Videometer Lab (B) Minolta chromometer versus Videometer Lab and (C) HunterLab instrument versus Minolta chromometer. It should be noted that HunterLab L*-value measurement for carcass 187 is an outlier.

Table 9 summarises the fresh meat colour measurements of bloomed LL and SM muscle using the Videometer Lab, Minolta chromameter and HunterLab instrument.

Table 9: Summary of fresh meat colour measurements using the Videometer Lab, Minolta and HunterLab colour meters on LL and SM muscle after 30 minutes bloom.

	LL			SM		
	L*	**a***	**b***	**L***	**a***	**b***
Videometer Lab						
Mean	30.82	19.68	12.35	30.35	18.53	11.76
SD	1.57	1.02	1.29	1.57	1.28	1.67
Highest value	34.34	24.39	20.97	34.01	22.62	19.76
Lowest Value	25.26	17.02	10.06	25.65	15.47	8.50
Minolta						
Mean	35.73	18.15	9.26	34.80	18.62	8.81
SD	1.86	1.52	1.29	1.78	1.37	1.14
Highest value	40.24	29.93	11.99	39.59	22.88	11.98
Lowest Value	21.69	13.03	4.74	27.98	14.15	5.32
HunterLab						
Mean	34.14	17.93	16.01	32.16	18.40	16.18
SD	2.43	1.34	1.36	2.29	1.17	1.31
Highest value	40.24	20.65	19.78	41.89	21.03	19.77
Lowest Value	19.62	8.40	8.49	23.03	15.21	11.21

<u>VISNIR regression models developed for VISNIR spectra obtained on a hot carcass for predicting Videometer Lab bloomed fresh meat colour L*-value:</u>

The first few PLS regression models developed revealed that pH recorded 30 minutes post-slaughter and temperature made no positive contribution to the models for predicting ultimate fresh meat colour. Figure 16 shows the best achievable Pencil probe regression model for predicting Videometer Lab L*-value (brightness).

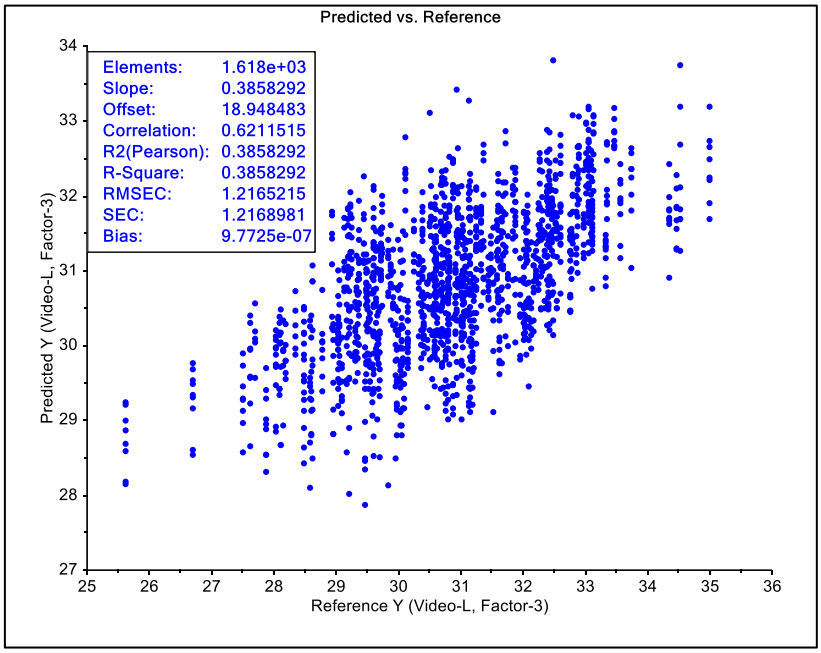


Figure 16: Three-factor PLS regression model developed for predicting Videometer Lab L*-value (i.e. ultimate brightness). Due to the large number of samples and the low number of factors, the RMSEC and RMSEP are almost identical.

During VISNIR PLS regression modeling, approximately 3% of the measurements were removed as outliers. This was considered acceptable because when the VISNIR spectra was acquired, there was no way to determine whether the spectra originated from muscle or other tissue type. Only in cases where VISNIR spectra was obviously not originating from a muscle surface at time of collection were the VISNIR spectra measurement repeated.

The 3-factor PLS regression model utilised only 38% of the spectral variation. However, unlike PCA regression models, it is not the objective of PLS regression models to describe the x-variation with as few factors as possible. PLS regression models seeks to model that part of the VISNIR spectra that contains useful information about the y-variable of interest (in this case L*-value, brightness). The model uses only 3 factors offering the promise of robustness.

By looking at the β-coefficients of the PLS regression model (i.e. the numbers that were multiplied on to the spectra values for predicting the L*-value); these values were of the expected magnitude (Table 10). This was because the L*-values from the Minolta, HunterLab and Videometer Lab are calculated from the green part of the spectra meaning that the PLS regression model developed should correlate positively with the green part of the spectrum. Whilst, we should observe the opposite effect for the red part of the spectrum where there is a negative correlation.

Table 10: β-coefficients for the visible region of the spectrum that were multiplied onto the VISNIR spectra values.

Wavelength (nm)	405	409	506	509	510	511	567	568	569	570
β-coefficient	0.020	-0.040	-0.026	0.031	0.035	0.032	0.035	0.036	0.034	0.030
Wavelength (nm)	625	626	627	628	629	636	637	684	685	690
β-coefficient	0.018	-0.019	-0.019	-0.017	-0.016	-0.022	-0.021	-0.016	-0.020	0.021

The results for predicting Videometer Lab L*-value in the SM muscle are shown in Figure 17. The PLS regression models developed for the Minolta Chromameter and HunterLab instrument for L*-value (i.e. ultimate brightness) are equally modelled as the Videometer Lab.

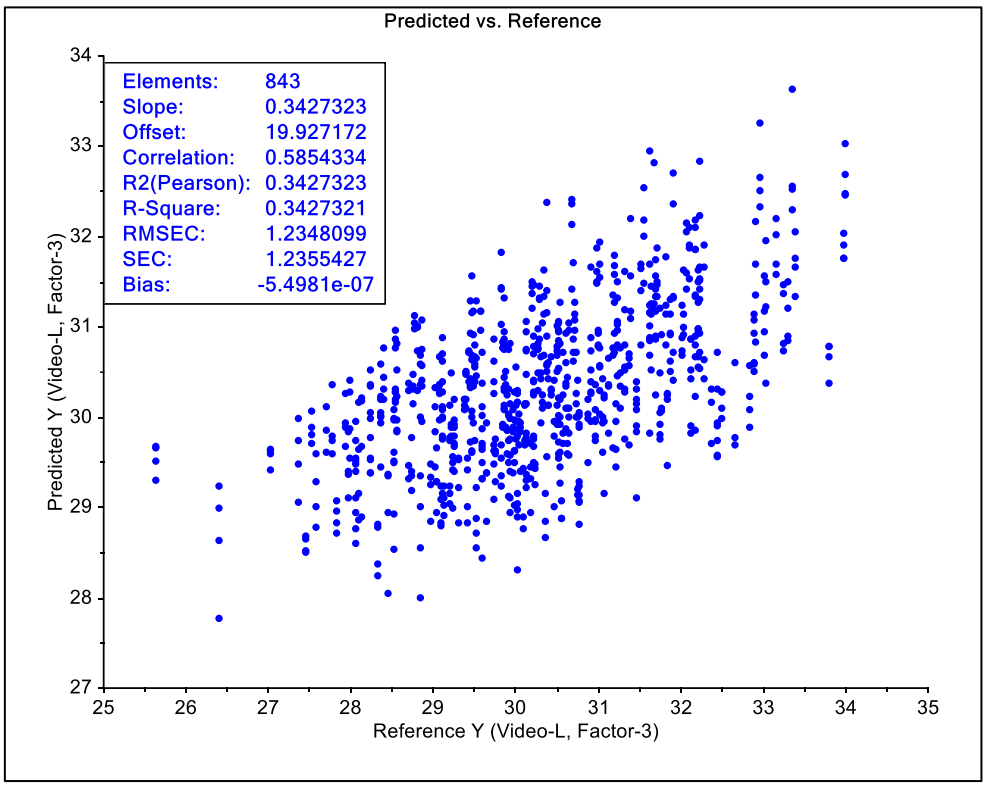


Figure 17: VISNIR PLS regression model for predicting L*-value of SM muscle. Due to the large number of samples and the low number of factors, the RMSEC and RMSEP are almost identical.

<u>VISNIR regression models developed for predicting HunterLab fresh meat colour L*-value 24 hours post-slaughter on a hot carcass:</u>

LL muscle: Using the pencil probe coupled with the ASD TerraSpec 4, VISNIR spectra were acquired on a hot carcass (i.e. 30 minutes post slaughter) on LL and SM muscle samples. Twenty four hours post slaughter, the brightness (L*-value) of bloomed LL and SM muscles were then measured using the HunterLab colour measurement instrument. Partial least squares (PLS) regression models were then developed to determine the effectiveness of VISNIR spectra obtained on a hot carcass at predicting fresh meat colour of fully bloomed LL and SM muscle at 24 hours post slaughter.

Using individual VISNIR spectra obtained on hot carcasses, the first PLS regression model developed combined wavelength regions 400nm – 980nm and 1020nm – 1250nm and predicted fresh meat colour of fully bloomed LL muscle samples (i.e. HunterLab L*-values). This results of which are shown in Figure 18. Further analysis revealed that averaging predictions over VISNIR spectra acquired in the LL muscle resulted in a reduction in the RMSEP (Figure 19).

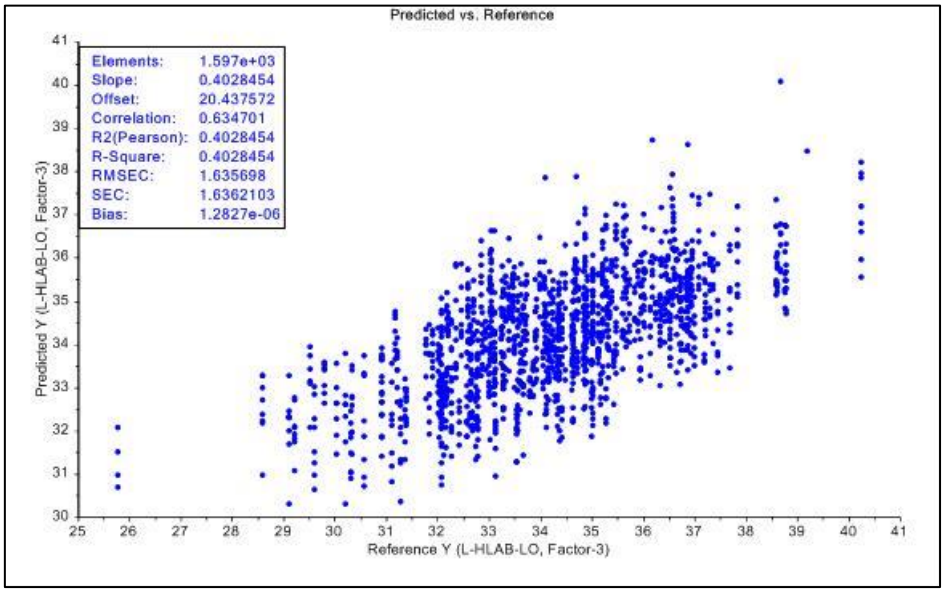


Figure 18: PLS regression model developed using individual VISNIR spectra acquired on a hot carcass at predicting HunterLab L*-value of bloomed LL muscle measured 24 hours post-slaughter. Cross validation rendered a correlation of R = 0.63 and RMSEP = 1.67 L*-value units.

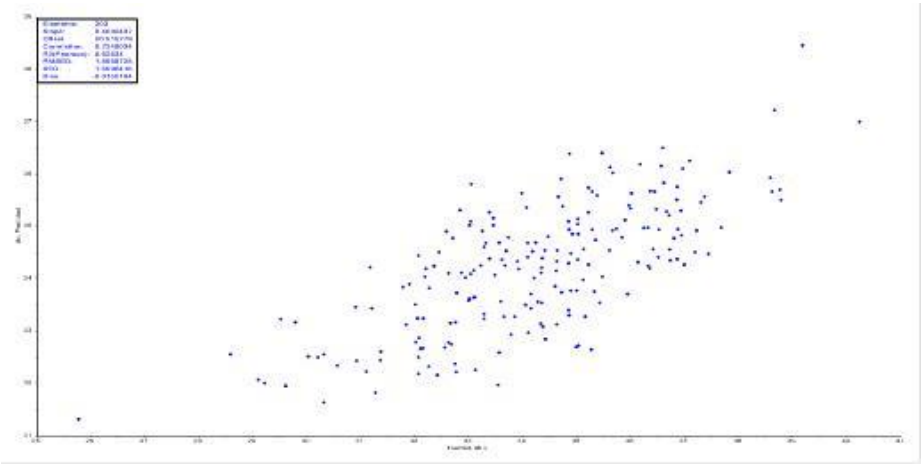


Figure 19: Predicted HunterLab L*-values using PLS regression model developed, averaged over VISNIR insertions per LL muscle (R = 0.72, RMSEP = 1.5558).

Classifying carcass in to 2 grading categories according to HunterLab L*-values:

A threshold L*-value of 34 was applied for the purpose of sorting carcasses in two grading categories – perceived consumer dark colour (L*-value < 34.0) and light colour (L*-value > 34.0); determined by consumer satisfaction preference studies [9]. Table 11 shows the distribution of predicted HunterLab L*-values using PLS regression model developed versus reference HunterLab L*-value measurements. The sensitivity and specificity of the PLS regression model developed was 72% and 28%, respectively. Sensitivity (i.e. the true positive rate) measures the proportion of positives are correctly identified (i.e. true positives, TP) over the total number of positives in the test (i.e. TP plus true negatives, TN). Whereas, specificity measures the proposition of negatives that are correctly identified (i.e. false positives, FP) over the total number of negatives in the test (FP plus false negatives, FN). It should be noted that 2 carcasses were removed from the analysis as they were either a VISNIR spectral or HunterLab colour measurement outlier.

Table 11: Sorting carcasses according to actual and predicted HunterLab L*-value classification greater or less than 34 (N= 203).

	Predicted L*-value	
Actual L*-value	**< 34**	**> 34**
< 34	55 (TN)	24 (FP)
> 34	34 (FN)	90 (TP)

TN = true negative, TP = true positive

FP = false positive, FN = false negative

Thus, the sensitivity and specificity for sorting carcasses according to actual and predicted classification for Hunter Lab L*-value is 73% and 70%, respectively.

SM muscle: Unfortunately, analysis VISNIR acquired spectra 30 minutes post-slaughter using the pencil probe on SM muscles from did not result in useful PLS regression models for classifying carcasses according to expected ultimate HunterLab L*-value. This is most likely due to the fact that there was no way of knowing whether the pencil probe tip was facing a meat surface or collagen/bone material. However, there was no reason to believe that it is necessary to develop separate PLS regression models for measuring fresh meat colour in muscles within the same species of animal. If this is true, the PLS regression model developed on the LL muscle will be applicable to leg muscles.

Modelling the LL and SM muscle collectively: Modelling both the LL and SM muscles in a single step greatly increases the RMSEP for the LL muscle HunterLab L*-value measurements. Figure 20 depicts the VISNIR regression model for predicting bloomed fresh meat colour HunterLab L*-value in LL and SM muscle of prime lamb carcasses. Figure 21 shows the distribution of SM muscle HunterLab L*-values centered in the region between 32 and 54 units and that nearly all the data points above 37 units are nearly all associated with the SM muscle. It should be noted that no outliers were removed from these plots, thus further improvement can be made to this PLS regression model. An important thing to note was that this model was developed using the HunterLab LL in carcass L*-value measurement whereas all other models were developed using the HunterLab LL out of carcass measurement.

The encouraging part of this model was that the SM muscles were all classified as being above a chosen threshold of either L*-value of 34 (Minolta chromameter) or 37.8 (modelled

HunterLab L*-value). If the VISNIR regression model is run entirely on the SM muscle there was very little correlation with predicting L*-value of lamb.

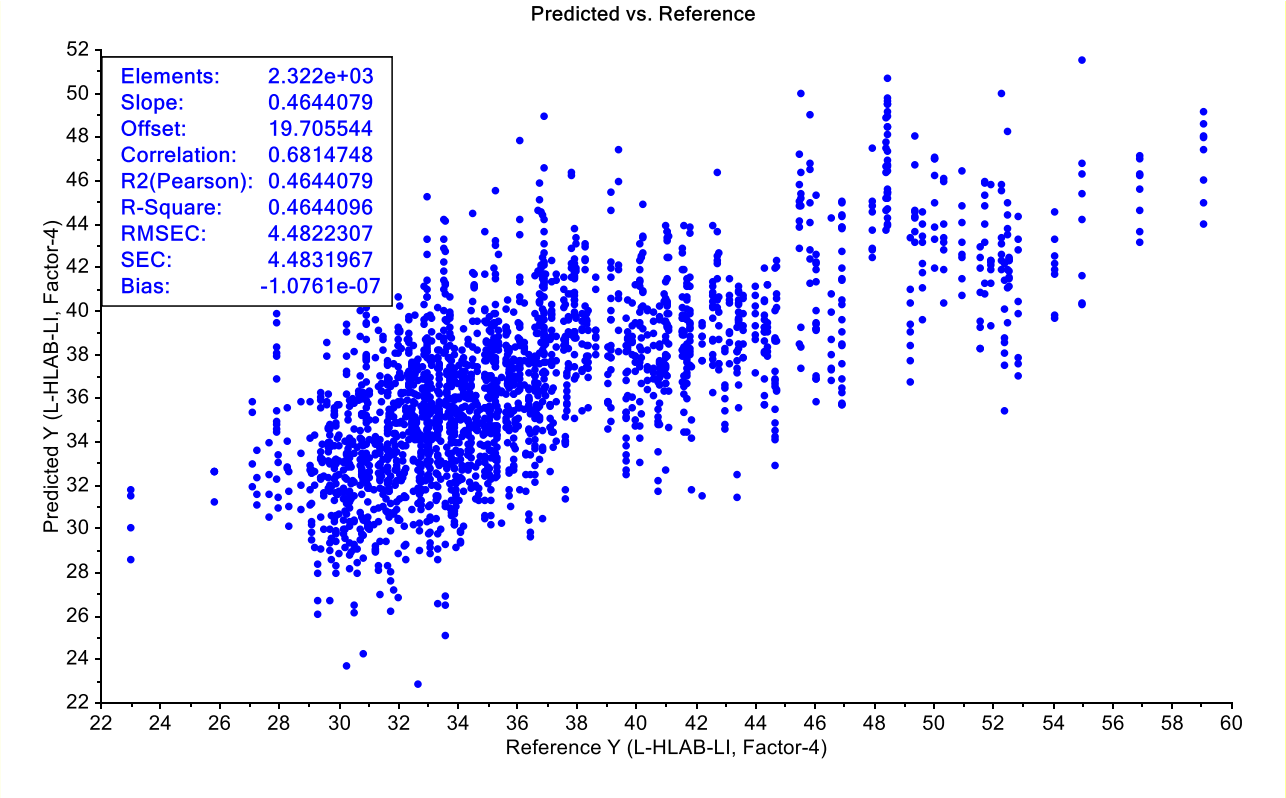


Figure 20: VISNIR regression model for predicting bloomed fresh meat colour HunterLab L*-value in LL and SM muscle of prime lamb carcasses.

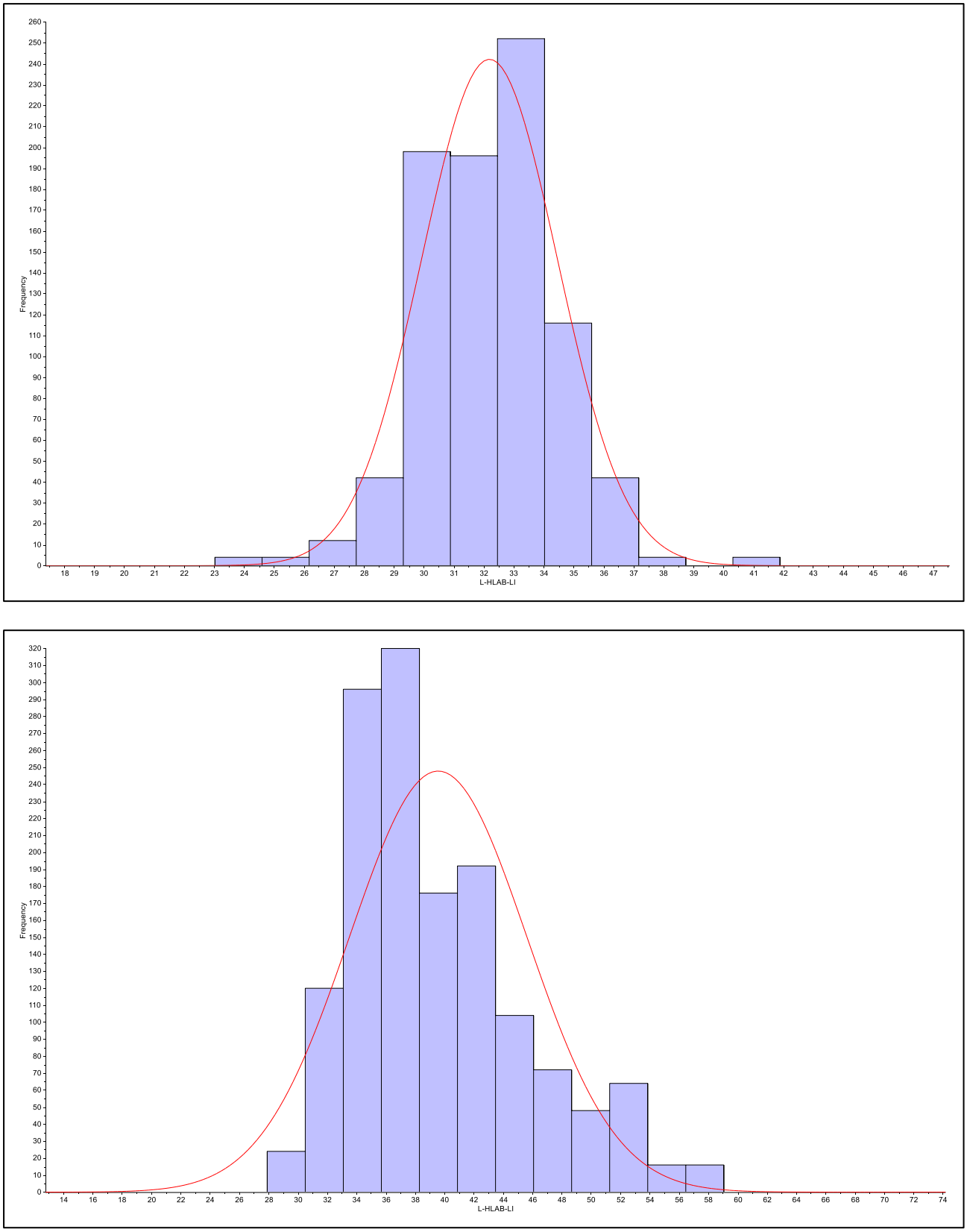


Figure 21: Distrubution of Hunter Lab L*-values for (A) LL and (B) SM muscles.

VISNIR regression models developed using spectra obtained 24 hours post slaughter for determining HunterLab L*-value

LL muscle measured in carcass: Twenty four hours post-slaughter, HunterLab L*-values and VISNIR spectra measurements were performed on the same cut meat surface in the carcass. Prior to HunterLab L*-value and VISNIR spectra acquisition, the cut surface was allowed to bloom for 30 minutes. VISNIR spectra were then acquired by the PAS handheld probe coupled with the ASD Labspec Pro. The PLS regression model for HunterLab L*-value was developed using 178 carcasses sampled in this study. This was because HunterLab L*-values were not collected for lambs slaughtered on day 1 of this study (i.e. carcasses 1 to 27). Figure 22 shows the predicted HunterLab L*-value versus the reference HunterLab L*-value for the PLS regression model developed. This PLS regression model was restricted to wavelength regions 400nm – 900nm and 1020nm – 1780nm. It should be noted that 5 individual data points were removed as outliers when developing this model.

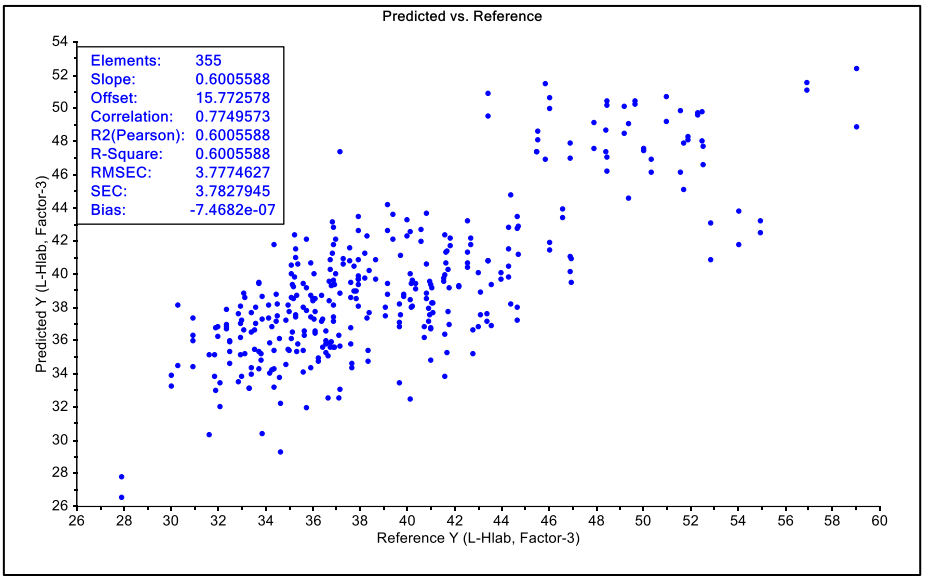


Figure 22: PLS regression model for predicting HunterLab L*-value for LL muscle measured in carcass using the PAS handheld probe coupled with the ASD Labspec Pro.

If the PLS regression model shown in Figure 22 is used for classifying carcasses according to Minolta L*-values being above or below 34, the HunterLab data firstly have to be bias corrected so the mean values of the HunterLab measurements match the mean L*-values measured by Minolta. For LL measurements in carcass, the mean Minolta L*- value was 35.72 and the mean HunterLab L*-value was 39.51. This means that we should set the threshold value at 37.79, if HunterLab L*-values are used.

Using a HunterLab L*-value of 37.8 as a threshold value for meeting satisfactory consumer preference, the sensitivity and specificity of the regression model developed is 89% and 67%, respectively. The distribution of predicted versus reference HunterLab L*-value results are shown in Table 12.

Table 12: Expected result for sorting according to expected Hunterlab L value greater or less than 37.79 (Minolta L = 34). N= 178.

	Predicted L*-value	
Actual L*-value	**< 37.8**	**> 37.8**
< 37.8	59 (TN)	29 (FP)
> 37.8	10 (FN)	80 (TP)

TN = true negative, TP = true positive
FP = false positive, FN = false negative

LL muscle measured out of carcass: HunterLab L*-values and VISNIR spectra measurements were performed on LL muscle samples removed from the carcass. The samples were allowed to bloom for 30 minutes, prior to VISNIR spectra measurement. VISNIR spectra was then acquired by the PAS handheld probe coupled with the ASD Labspec Pro. Two PLS regression models were developed for predicting HunterLab L*-values. The first model utilised wavelength regions between 400nm – 980nm and 1020nm – 1780nm; whilst the second model restricted the wavelength region to between 400nm – 700nm (i.e. the visible spectrum range). Figure 23 and Figure 24 shows the predicted HunterLab L*-value versus the reference HunterLab L*-value for both models. The prediction accuracy of model 2 deteriorates slightly when compared to model 1.

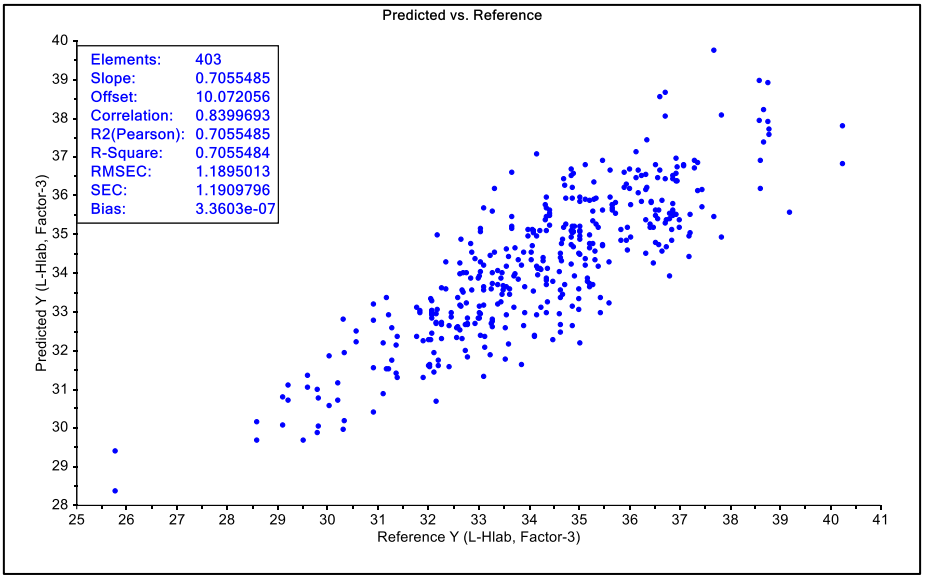


Figure 23: PLS regression model 1 using wavelength regions between 400nm – 980nm and 1020nm – 1780nm developed for predicting HunterLab L*-value for LL muscle removed from carcass using the PAS handheld probe coupled with the ASD Labspec Pro.

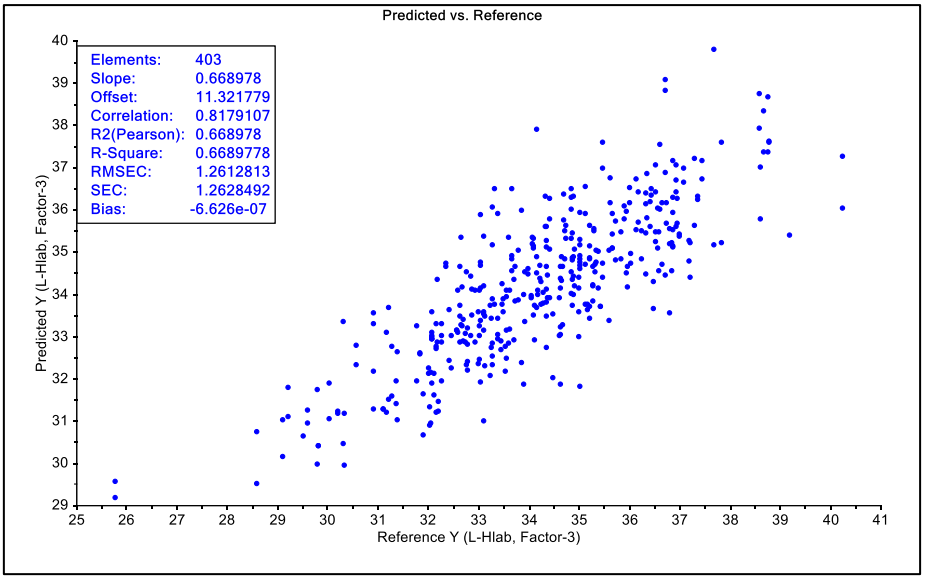


Figure 24: PLS regression model 2 developed using the visible spectrum range for predicting HunterLab L*-value for LL muscle removed from carcass using the PAS handheld probe coupled with the ASD Labspec Pro.

4.5 Meat tenderness (SF5)

In the loin muscle, shear force at day 5 of ageing ranged between 19.5 and 73.4 N. The mean SF5 was 34.2 ± 9.3 N (± SD) (see Table 3). Only 14 animals out of 205 animals had a SF5 greater than 50 N. Whilst in the topside muscle, shear force at day 5 ageing ranged between 23 and 76 N. The mean SF 5 was 51.5 ± 10.6 N (± SD). One hundred and twelve animals out of 205 animals had a SF5 greater than 50 N. Figure 25 shows the meat tenderness (SF5) on LL and SM muscles sampled from 205 lamb carcasses.

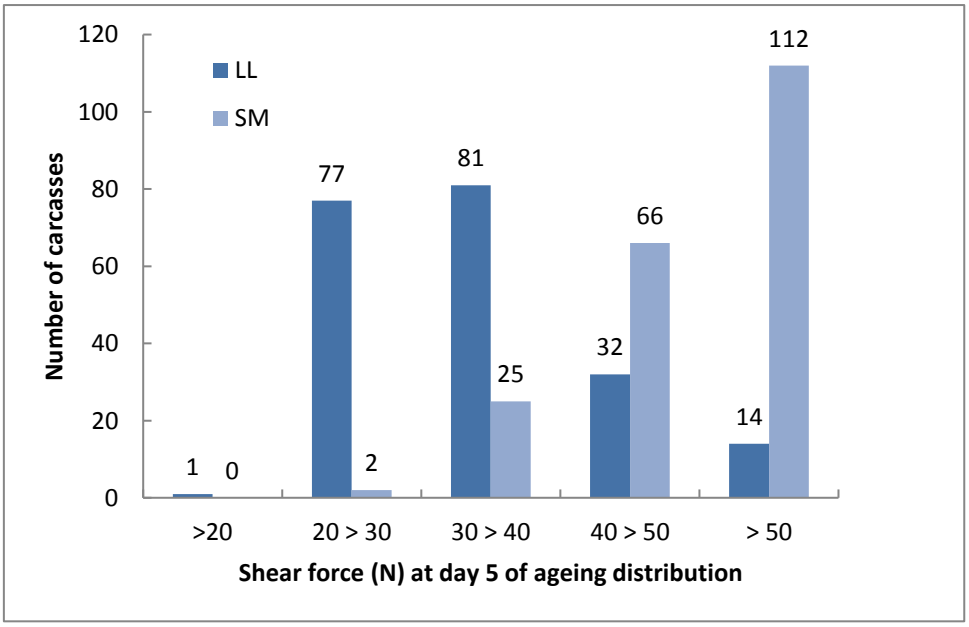


Figure 25: Distribution of meat tenderness (shear force at 5 days of ageing, SF5) on loin (LL) and topside (SM) muscle sampled from all 205 lamb carcasses.

Table 13 reveals the statistical interpretation for the VISNIR regression model developed for SF5 for both LL in and out of carcass and SM muscle using the PAS handheld probe instrument configuration, 24 hours post-slaughter. Individual VISNIR regression models were developed for LL in and out of carcass and SM muscles. These models were then used to classify carcasses at 2 different SF5 categories – 40N or 50N. Figure 26 is the scatter plot represents the VISNIR predicted SF5 measurment versus the reference SF5 measurment for LL muscle. Table 14 reveals the success of the LL and SM muscle VISNIR regression models at predicting SF5. The VISNIR regression model developed for SF5 had greater than 79% accuracy at predicting SF5 greater than 40N in LL muscle; whilst, in SM muscle the accuracy is reduced to 72% at 40N.

Table 13: Shear force at day 5 of ageing VISNIR regression model statistical interpretation for loin (LL) in and out of carcass and topside (SM) muscle. This VISNIR regression model was generated using the custom made PAS handheld probe coupled with the ASD Labspec Pro, 24 hours post-slaughter.

Shear force at day 5 of ageing	LL (in carcass)	LL (out of carcass)	SM
SECV	7.0	8.3	8.9
Uncertainty	±14.0	±16.6	±17.8
R-squared	0.34	0.20	0.30
Slope	1.35	1.16	1.20
Offset	-11.65	-5.44	-10.21
BIAS	0.35	0.25	0.05

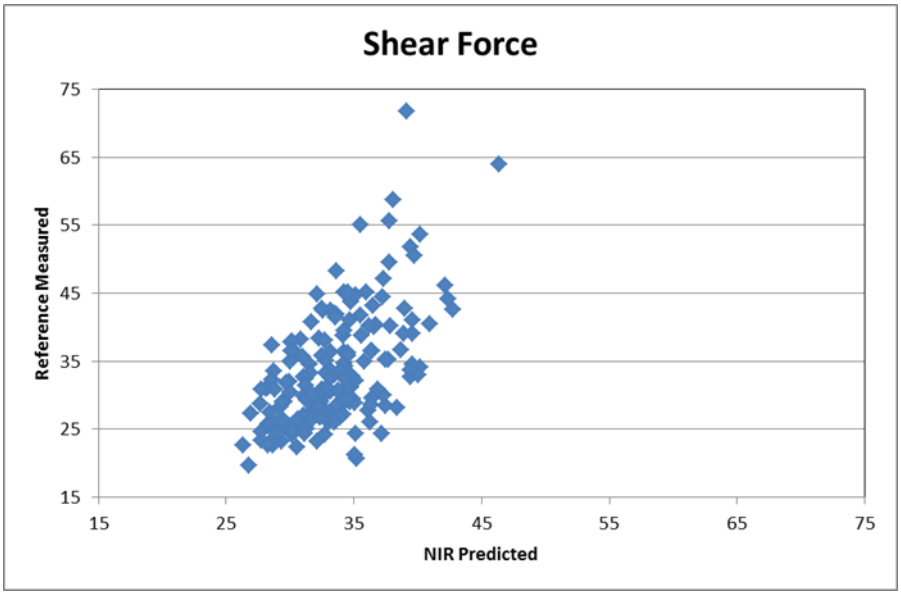


Figure 26: Scatter plot representing VISNIR predicted shear force at day 5 of ageing versus the reference measured shear force at day 5 of ageing for loin (LL) in carcass measurements.

Table 14: Success of VISNIR regression model at classifying carcasses into 2 different shear force at day 5 of ageing categories – 40N or 50N.

Shear force at day 5 of ageing	LL (in carcass)	LL (out of carcass)	SM
Cutoff (N)	50	50	50
Correctly Assigned	172	189	139
False Pass	8	14	31
False Fail	0	0	35
%Correctly Assigned	96%	93%	68%
%False Pass	4%	7%	15%
%False Fail	0%	0%	17%
Cutoff (N)	40	40	40
Correctly Assigned	148	161	178
False Pass	31	40	0
False Fail	1	2	27
%Correctly Assigned	82%	79%	72%
%False Pass	17%	20%	0%
%False Fail	1%	1%	13%

4.6 Intramuscular fat content

The mean IMF content for all lambs in this study was 3.3 ± 1.0% (± SD) and 3.2 ± 0.9% (± SD) in LL and SM muscle, respectively (see Table 3). Seventy six percent and 85% of LL and SM muscle had an IMF content lower than 4%, respectively. Figure 27 shows the distribution of IMF content of LL and SM muscle from all 205 lamb carcasses sampled.

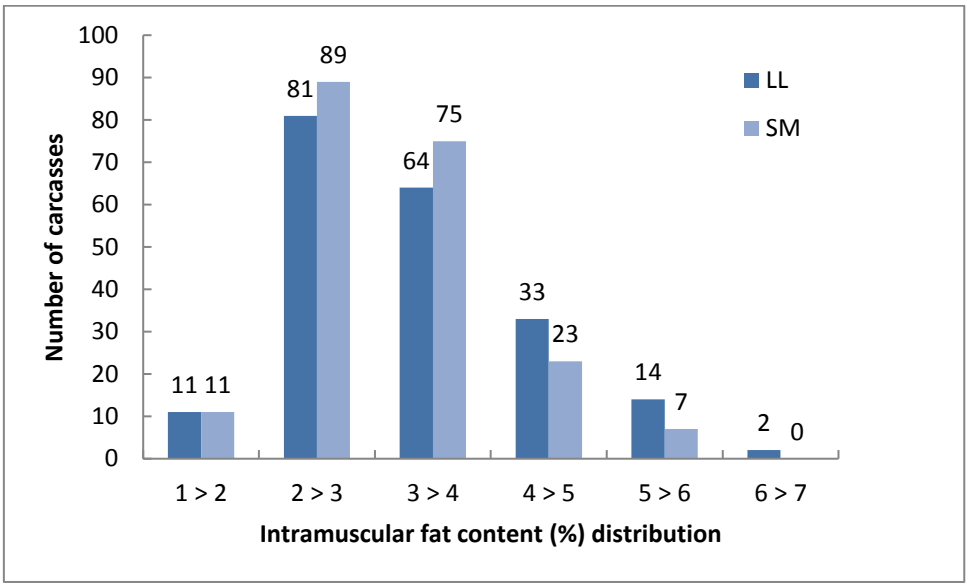


Figure 27: Distribution of intramuscular fat content (%) of loin (LL) and topside (SM) muscle from all 205 lamb carcasses.

Table 15 reveals the statistical interpretation for the VISNIR regression model developed for IMF content for both LL (in and out of carcass) and SM muscle using the PAS handheld probe coupled with the ASD Labspec Pro, 24 hours post-slaughter. Individual VISNIR regression models were developed for LL and SM muscles. These models were then used to classify

carcasses at IMF content of 3.5% based on consumer satisfaction results. Figure 27 is the scatter plot represents the VISNIR predicted IMF content versus the reference IMF content for LL muscle. Table 16 reveals the success of the LL and SM muscle VISNIR regression models at predicting IMF content. The VISNIR regression model developed for IMF content had greater than 78% accuracy at predicting IMF content of 3.5% in LL muscle; whilst, in SM muscle the accuracy is improved slightly to 80%.

Table 15: Intramuscular fat content VISNIR regression model statistical interpretation for loin (LL) in and out of carcass and topside (SM) muscle. This VISNIR regression model was generated using the handheld probe coupled with the ASD Labspec Pro, 24 hours post-slaughter.

IMF content	LL (in carcass)	LL (out of carcass)	SM
SECV	0.7	0.6	0.5
Uncertainty	±1.4	±1.2	±1.0
R-squared	0.55	0.60	0.63
Slope	1.29	1.26	1.19
Offset	-0.97	-0.87	-0.58
Bias	-0.25	-0.10	0.45

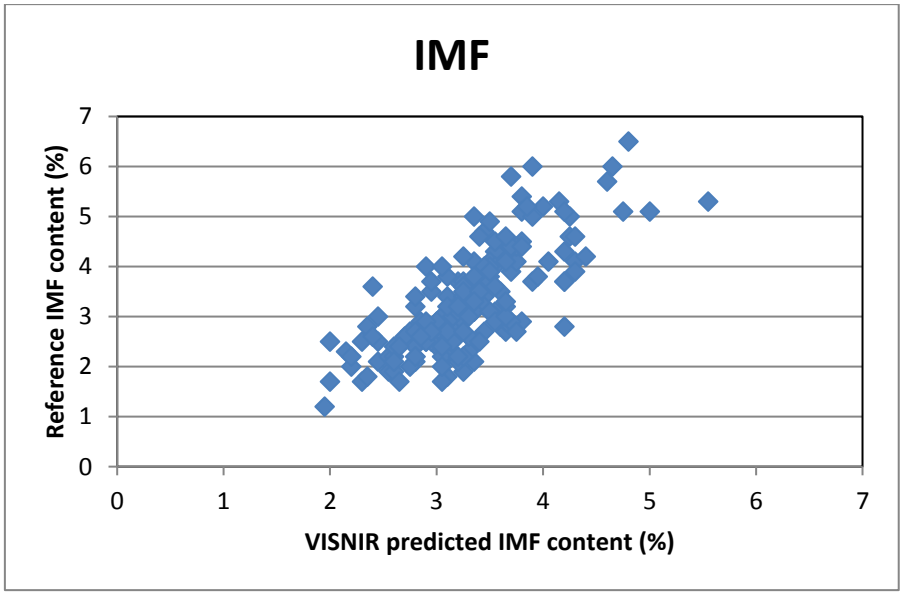


Figure 28: Scatter plot representing VISNIR predicted intramuscular fat content versus the reference measured IMF content for LL in carcass measurements.

Table 16: Success of VISNIR regression model predicting intramuscular fat content.

IMF content	LL (in carcass)	LL (out of carcass)	SM
Cutoff (%)	3.5	3.5	3.5
Correctly Assigned	147	172	186
False Pass	15	9	5
False Fail	18	22	14
%Correctly Assigned	82%	85%	91%
%False Pass	8%	4%	2%
%False Fail	10%	11%	7%

Table 17: Post rigour VISNIR validation models for predicting muscle ultimate pH (pHu), total glycogen content, intramuscular fat content and meat tenderness (SF5) from spectra collected on chilled lamb carcasses 24 hours post slaughter using the PAS handheld probe coupled with the ASD Labspec Pro spectrometer.

VISNIR Model	Reference /Validation model statisitics	pHu		Total glycogen content (μmol/g)		IMF content (%)		SF5 (N)	
Muscle		**LL**	**SM**	**LL**	**SM**	**LL**	**SM**	**LL**	**SM**
Calibration Model	Spectra	262	296	262	294	262	296	262	296
	N	131	148	131	147	131	148	131	203
	Mean	5.63	5.64	61.7	60.5	3.3	3.1	34.2	51.5
	Ref SEM[1]	0.088	0.104	2.621	1.862	0.114	0.092	4.746	6.848
	Ref CV (%)	1.6	1.9			3.7	2.9	13.9	13.3
	Calibration model SECV	0.1	0.1	11.9	10.2	0.85	0.75	8.5	10.1
	Calibration model R^2	0.22	0.37	0.03	0.15	0.36	0.21	0.10	0.12
	PLS factors	5	7	5	5	6	7	3	4
Validation Model	Validation (N)	49	48	48	46	48	48	49	48
	Validation model SEP	0.2	0.2	11.6	9.9	0.6	0.8	7.2	10.0
	Validation model R^2	0.39	0.46	0.39	0.33	0.38	0.29	0.13	0.12
	Nominated cut off value	5.7	5.7	40	40	3.5	3.5	50	50
	Model classification accuracy[2] (%)	94	96	83	91	88	77	98	70

[1]Reference method standard error of measurement (i.e. repeatability of the standard reference measurement).

[2] Percentage correctly assigned above or below nominated cut-off value.

5 Discussion

Consumers expect premium quality and value for money when purchasing prime lamb meat [10]. To meet these consumer expectations, there is a need for the Australian sheep meat industry to develop objective carcass measurement technology that is able to accurately predict meat quality. The objective carcass measurement technology must be robust, reliable and cost effective and be able to grade prime lamb carcasses at chain speed. This project evaluated the application of VISNIR technology at predicting lamb product quality. To achieve this goal, VISNIR calibration and validation models were developed for ultimate pH, glycogen content, fresh meat colour, meat tenderness and intramuscular fat content.

5.1 Ultimate pH

Ultimate pH is one of the technical attributes that drives consumers purchasing decisions of meat [11]. The variation in ultimate pH influences factors such as colour and the ability of the meat to retain water. A low ultimate pH results in meat proteins having decreased water-holding capacity and a lighter colour. While a high ultimate pH will give a darker colour and less drip loss and is often associated with poor eating quality of lamb, beef and pork [12, 13]. Ultimate pH of meat is conventionaly measured in the abattoir using a pH meter. The pH meter measures changes in proton (H^+ ion) concentrations and is often a slow and laborious process. Therefore, the development of a VISNIR calibration and regression model that is able to classify the ultimate pH will greatly assist in improving the current classification for Australian lamb.

The relationship between predicted VISNIR regression model ultimate pH values and observed ultimate pH values in lamb were moderate (Table 4, R^2 ranging from 0.49 - 0.7). These findings were in agreement with previous VISNIR regression models developed for beef (R^2 = 0.62, De Marchi, *et al.* [14]), lamb (R^2 = 0.26, Andres, *et al.* [15]) and pork (R^2 = 0.67, Hoving-Bolink, *et al.* [16]). Many authors believe the observed relationship may be a result of the reference data having a limited pH range [17]. For example, in this study, the mean ultimate pH was 5.63 for LL muscle; with the maximum and minimum observed ultimate pH of 6.76 and 5.41 units, respectively (Table 3). We observed similar ultimate pH values for the SM muscle. Another factor that contributes the moderate relationship between predicted and observed ultimate pH values is the fact that VISNIR technology will indirectly measure the ultimate pH of meat by measuring the stretching/distortion in hydroxyl bonds (-OH bonds). However, Savenije, *et al.* [18] believed measuring the ultimate pH of meat indirectly was not an issue. These authors showed that VISNIR predicted the ultimate pH within 0.1 pH unit for 84% of meat samples.

Also, previous research using a restricted ultimate pH data set identified a a stronger relationship (R^2 = 0.89) between predicted VISNIR spectra pH_{45min} and observed pH_{45min} post-slaughter [15]. These findings suggest changes in pH associated with early post-mortem lead to greatest variation in VISNIR spectral data. Noting this observation by Andres, *et al.* [15], we believed developing a VISNIR regression that predicted the observed pH_{45min} post-slaughter was not a practical measure for grading Australian lamb carcasses. This was because of variation in the age of the animal at slaughter, carcass weight and the body composition would contribute significantly to the rate of pH decline at 45 mins post-slaughter. Therefore, developing a VISNIR regression at 24 hours post-slaughter may reduce this

observed variation. Therefore VISNIR is a suitable alternative to the pH meter which is slow and has low precision under routine operation [18].

We observed a moderate correlation between actual and predicted ultimate pH of individual lamb carcasses, a calibration and validation regression model was developed with the aim of classifying the ultimate pH of LL and SM muscle from individual lamb carcasses at 5.7 or below; an acceptable target for lamb to ensure consumer sensory satisfaction and acceptable eating quality. Based on the SECV estimates and classification accuracies of the VISNIR prediticiton and validation models developed for ultimate pH (Table 4 and 5), these results suggest VISNIR technology could be used to classify individual lamb carcasses into high or low ultimate pH categories at 24 hours post slaughter. Our results are not as precise as Cozzolino and Murray [19] and Andres, *et al.* [20] who accurately predicted the ultimate pH of beef samples. This was probably due to our data having a narrow pH range in the reference data. However, like Cozzolino and Murray [15] and Andres, *et al.* [20], we had good repeatability of the reference method and the VISNIR instrument was presented to intact meat in the carcass.

We propose to capture individual carcasses that were incorrectly classified with a high ultimate pH; we suggest meat processors should retest these carcasses with a pH meter to verify their classification status. Any carcasses that are falsely classified for high ultimate pH could then be returned returned to the appropriate ultimate pH cohort. This would ensure a more thorough appraisal of ultimate pH across carcasses and provide a more reliable, efficient and rapid MSA grading outcome for ultimate pH.

To further improve the VISNIR calibration and prediction model for ultimate pH, additional work is required to determine whether breed, age and finishing diet (pasture or grain) impacts on the accuracy of prediction of ultimate pH when classifying animals above or below the ultimate pH threshold.

5.2 Glycogen content

The total glycogen content or the glycolytic potential (GP is the sum of all glycolytic metabolites) of muscle remains constant from slaughter to rigour. In theory, this allows muscle glycolytic metabolite levels to be reliably calculated at any nominated time point from slaughter to 24 hours post slaughter. Using this knowledge and the total glycogen content of meat determined at 30 mins post-slaughter, a VISNIR regression model was developed to predict the ultimate pH of meat at 24 hours post-slaughter using VISNIR spectra obtained using the pencil probe at 30 mins post-slaughter and the level of pre-rigor muscle glycolytic metabolites. The aim was to develop a VISNIR regression model capable of predicting the ultimate pH of meat using “hot” carcasses on the kill floor. Unfortunately, this was not achievable (data not shown).

A second VISNIR model was developed to predict the muscle total glycogen levels at 30 minutes from VISNIR scans collected at 24 hours post slaughter. The model developed was able to predict the total glycogen content of meat with 83% accuracy at above or below a nominated cut off value of 40 μmol/g.

5.3 Fresh meat colour

Meat colour is closely associated with the customer's first appraisal to lamb [4]. Previous research has established consumers willingness to pay a premium for red meat [21]. Many consumers deem fresh meat colour as a visual measure of freshness and quality [22]. To evaluate fresh meat colour, there are several assessment methods; visual assessment, use of reference (beef grading) colour chips or instrumentation [9].

The relationship between 2 established colour instruments (the HunterLab and Minolta chromameter instruments) and the Videometer Lab instrument against beef reference colour chips was established. Figure 11 showed a close relationship existed between the dynamic range of the *a**- and *b**-value beef grading colour chip measurement for the Hunter Lab and Videometer Lab instrument. Whilst the lightness (L*-value) of the Minolta chromameter and Videometer Lab instrument had a similar dynamic range. For Australian lamb, previous research by Hopkins [23] showed that when the brightness of meat (L*-value) dropped below 35, consumers considered the meat was unacceptably dark and a redness value (a*) below 19. Using beef reference colour chips, the lightness of the reference beef colour chip did not drop below 35 for the Minolta chromameter or the Videometer lab instrument. Whilst the HunterLab instrument dropped below 35 for the majority of beef reference chips. The redness of the beef reference colour chips was below a threshold of 19 for the majority of beef grading chips whereas the HunterLab instrument and the Videometer lab was above the threshold of 19 (Figure 11). The variation observed was possibily due to differences in lighting conditions and the angle of detection for each instrument. These parameters are likely to vary between all colorimetric devices. This result is an important finding considering several hyperstpectral devices are currently being developed to determine meat quality traits. It shows the need to develop an indepth understanding of the relationship between the hyperspectral devices (in this study - Videometer Lab) and traditional colourimetric devices (Minolta Chromameter or HunterLab instruments) when determining the relationships with consumer acceptablility preferences and product quality (i.e. fresh meat colour and its alignment with consumer acceptability preferences).

Figure 13 details visual assessment of meat samples using beef grading colour chips determined by MSA graders and the variation observed in HunterLab L*-values (lightness of meat). The observed variation was not anticipated, however, this result was probably expected because the visual assessment of meat is a subjective measurement of overall meat colour whereas the lightness of meat determined by the HunterLab instrument is entirely dependent on the location of the aperture on the meat surface. Also, the Hunter Lab instrument is also unable to discriminate between intramuscular fat content and meat. These results highlight the technical challenges associated with objectively measuring fresh meat colour using a sole reference standard (i.e. beef reference colour chips) and MSA graders. Previous work in beef identified that VISNIR calibration/validation models developed using MSA grader reference colour chip assessment of meat colour were unsuccessful (A.MQT.0052). This study reports similar findings for lamb.

The application of VISNIR technology to predict meat L*, a* and b* colour values measured by a Minolta colourimeter has been widely evaluated by the meat industry [16, 18-20, 24]. However, these results have often been contradictory [17]. This study showed that reducing the region of the visible spectra analysed (green area of the spectra, Table 10 – 509nm – 511nm and 568nm - 570nm), VISNIR regression models could be developed to predict the

brightness of meat (L*-value) of meat determined by the Videometer Lab using VISNIR spectra obtained using the pencil probe at 30 minutes post-slaughter (Figure 16 and 17, LL and SM muscle, respectively).

To assess the practical utility of the prediction model, the ratio performance deviation (RPD) and the range error ratio were determined (RER). The RPD is the ratio of SD to RMSEcv of a given trait [25] and the RER is the ratio of the range to the RMSEcv of the trait [14]. A RPD value greater than 10 is considered equivalent to the reference methodology [26] and for analytical purposes a value greater than 2.5 is adequate [27]. The RER is a method for standardising the $RMSE_{CV}$ by relating it to the range of the reference data; RER values less than 6 indicate very poor classification; RER values between 7 and 20 classify the model as poor to fair and indicate it could be used for screening purposes; and RER values between 21 and 30 indicate a good classification suggesting the model would be suitable for application in quality control [28]. For LL muscle, the RPD and RER for the Videometer Lab VISNIR prediction were 1.29 and 7.46, respectively. Therefore the model developed was therefore considered poor to fair for predicting the brightness of meat (L*-value) using the Videometer Lab. A similar model was developed to determine the L*-value of LL muscle determined by the HunterLab colour instrument using VISNIR spectra collected using the Pencil probe at 30 mins post-slaughter. The RPD and RER for predicting the L*-value of meat determined by HunterLab were 1.48 and 12.60, respectively. The accuracy of this model is approaching the accuracy required to predict the L*-value of LL muscle of meat. The model slightly improves if the VISNIR spectra are averaged prior to developing the regression model (Figure 19), the RPD and RER of 1.56 and 13.22. Similar models were observed for predicting the L*-value of SM muscle. These results are equivalent to those reported in intact beef muscle [14].

Noting the size of the pencil probe and the ability the limited ability to develop regression models to predict fresh meat colour (L*-, a*- and b*-values), VISNIR spectra were collected on another device (PAS handheld probe). However, collectively modelling LL and SM muscle in one regression model increased the accuracy of prediction and classification to predict HunterLab L*-value in LL muscle in carcass. This was achieved using VISNIR spectra collected with the pencil probe at 30 minutes post-slaughter. The improved accuracy of prediction of HunterLab L*-value in LL was because the distribution of the reference measurement increased, ranging from 23 to 58 units (Figure 21) when incorporating LL and SM muscles. Several models developed used VISNIR spectra obtained using the PAS handheld probe at 24 hours post-slaughter (Figures 22, 23 and 24). When classifying the HunterLab L*-value of carcasses, the sensitivity and specificity of the regression models developed were 89% and 67%, respectively (Table 12).

These overall findings have improved our understanding of the factors influencing VISNIR's ability to measure fresh meat L*-, a*- and b*-colour. However, further improvement in all model accuracies are required before VISNIR could be reliable used as a grading tool to support MSA grading of lamb for fresh meat colour.

5.4 Meat tenderness

To meet consumer acceptability for Australian lamb, meat tenderness of approximately 27 N determined by shear force after 5 days ageing is required [29]. Pannier, *et al.* [30], showed that shear force at 5 days of ageing above this threshold had a negative relationship with all consumer sensory scores (i.e. overall liking, juiciness, tenderness, flavour, odour and taste of lamb). Prieto, *et al.* [17] showed that meat tenderness determined by shear force is closely related to the chemical composition of meat.

The main objective of this study was to assess the on-line implementation of VISNIR technology at 24 h post-mortem at predicting meat tenderness of lamb after 5 days of ageing at between 2 – 3ºC. Noting that after 5 days ageing, several biochemical and structural changes arise affecting meat tenderness [31] and the meat sample presented to the VISNIR instrument differed from the meat sample used to determine meat tenderness. Irrespective of these differences, the VISNIR calibration and validation models reported for meat tenderness from VISNIR sprectra collected at 24 hours post slaughter are encouraging. Two nominated cut off values for meat tenderness were set when determining the correlation and classification accuracies of prediction – 40 N and 50 N. The reason for selecting the nominated cut-off value were because previous VISNIR studies and the distribution of SF5 values reported in this study (Table 3). Whilst the R^2 values of the models are low (R^2 ranging from = 0.1 to 0.37) and the classification accuracies above or below nominated cut off value are encouraging and indicate there is valuable information in the spectra associated with this trait. Comparable results have been reported for beef [32]. However, their attempt to comercialise this application were unsuccessful.

5.5 Intramuscular fat content

The VISNIR validation models reported for tenderness and intramuscular fat level from VISNIR scans collected at 24 hours post slaughter are also encouraging. Whilst the R^2 values of the models are low to moderate the classification accuracies above or below nominated cut off values are encouraging and indicate there is valuable information in the spectra associated with both of these traits. This result is not surprising for intramuscular fat content where similar NIR based applications measuring fat level are well established in other sectors of the food industry. The result is more surprising for shear force although Shackleford & Koomarhrie have reported comparable results for beef. Their attempt to commercialise this application was reportedly unsuccessful.

IMF content influences the consumer's palatability of lamb meat [30, 33, 34]. Previous research has clearly identified that the IMF content contributes to the overall liking, juiciness, tenderness, flavour, odour and taste of lamb. In beef, Dikeman [35] showed that IMF content was associated with a 10 – 15% variance in consumer palatability of meat. Whilst in lamb meat, a threshold of 4 – 5% IMF content is required to achieve consumer palatability [29]. Pannier, *et. al.* [30] showed that an IMF content greater than 4.5% improved the consumer overall liking sensory score of lamb in LL and SM muscle 10 and 6.6 units, respectively. Previous research has identified that for every 1% increase in IMF content, an increase in consumer sensory satisfaction range of overall liking of lamb ranged between 1.23 units [33] and 2.2 units [30]. Pleasants, Thompson and Pethick [36] showed that consumer sensory scores must exceed 70 units for consumers to rate a cut of lamb better than everyday quality.

These authors promote a consumer sensory score greater than 70 should be the goal for the Australian lamb industry.

6 Recommendations

The key findings described in this Final Report support progressing the project to the third and final commercialisation phase. The commercialisation phase will focus on:

- Validation of VISNIR prediction models:
  Further validation of post rigor VISNIR prediction models for muscle glycogen, ultimate pH, meat tenderness (i.e. SF5) and intramuscular fat percentage. This will involve testing the VISNIR prediction models developed on a wider cross section of lambs processed at 2 different meat processing plants.

- VISNIR instrumentation and software commercialisation:
  Develop commercial partnership with Portable Analytical Solutions (PAS, www.portableas.com) to create VISNIR instrumentation and software that is compatible with the meat industry requirements. This includes:
  1. ensuring the VISNIR instrumentation and software are suitable for application in the meat processing sector (i.e. portability, operator ease of application, robustness and reliability), and
  2. meets Aus Meat MSA grading requirements for lamb.

- VISNIR technology to support beef MSA grading:
  The success of VISNIR technology at predicting intramuscular fat percentage of lamb provides a great opportunity for the beef industry. We propose that once VISNIR instrumentation and software are commercially developed for application in the lamb industry, this technology should then be trialled on MSA grading intramuscular fat content of beef carcasses. VISNIR regression and validation models for intramuscular fat content in beef will then be compared with traditional beef marbling scores. If VISNIR methodology proves successful, the beef industry will be able to develop an objective measure of beef marbling that is reliable and quantifiable.

7 Key Messages

This final report details the potential of VISNIR technology at predicting intramuscular fat content of lamb using a hand held probe at 24 hours post-slaughter. We understand that further refinement is required prior to application in the meat processing sector and accreditation by Aus Meat under Quality Management Systems: ISO 9001:2015. However, the application of this technology in the food and beverage sector means the development of commercial applications and quality assurance protocols for this specific application is achievable. Also, the ease of application, will enable fast tracking the development of training packages and implementation in industry.

8 References

1. Anon., ed. *AUS-MEAT language*. Fourth Edition ed. 1992, Authority for Uniform Specification Meat and Livestock: Sydney.
2. Anon., *Handbook of Australian Sheepmeat Processing*. 2015, Murarrie, Qld.: AUS-Meat Limited.
3. McPhail, N.G., et al., *Factors influencing the occurrence of high ultimate pH in three muscles of lamb carcasses in Australia.* Animal Production Science, 2014. **54**(10): p. 1853-1859.
4. Holman, B.W.B., et al., *Lamb meat colour values (HunterLab CIE and reflectance) are influenced by aperture size (5mm v. 25mm).* Meat Science, 2015. **100**(0): p. 202-208.
5. Warner, R.D., et al., *Quality of lamb meat from the Information Nucleus Flock.* Animal Production Science, 2010. **50**(12): p. 1123-1134.
6. Hopkins, D.L. and J.M. Thompson, *The relationship between tenderness, proteolysis, muscle contraction and dissociation of actomyosin.* Meat Science, 2001. **57**(1): p. 1-12.
7. Savell, J.W., H.R. Cross, and G.C. Smith, *Percentage Ether Extractable Fat and Moisture Content of Beef Longissimus Muscle as Related to USDA Marbling Score.* Journal of Food Science, 1986. **51**(3): p. 838-839.
8. Coombes, S.V., et al., *The impact of beef cattle temperament assessed using flight speed on muscle glycogen, muscle lactate and plasma lactate concentrations at slaughter.* Meat Science 2014. **98**(4): p. 815-821.
9. Khliji, S., et al., *Relationship between consumer ranking of lamb colour and objective measures of colour.* Meat Science, 2010. **85**(2): p. 224-229.
10. Pethick, D.W., et al., *Australian prime lamb - A vision for 2020.* International Journal of Sheep and Wool Science, 2006. **54**(1): p. 66-73.
11. Klont, R., *Influence of ultimate pH on meat quality and consumer purchasing decisions*. 2005, The Pig Site: http://www.thepigsite.com/articles/1506/influence-of-ultimate-ph-on-meat-quality-and-consumer-purchasing-decisions/.
12. Devine, C.E. *Incidence of high pH beef and lamb. I: implications for meat quality.* . in *Proceedings Meat Industry Research Conference*. 1994. Auckland, New Zealand
13. Channon, H. and R. Warner. *Delivering consistent quality Australian pork to consumers – a systems approach.* in *Australasian Pig Science Association Inc.* 2011. Werribee, Victoria.
14. De Marchi, M., et al., *The relevance of different near infrared technologies and sample treatments for predicting meat quality traits in commercial beef cuts.* Meat Sci, 2013. **93**(2): p. 329-35.
15. Andres, S., et al., *Prediction of sensory characteristics of lamb meat samples by near infrared reflectance spectroscopy.* Meat Sci, 2007. **76**(3): p. 509-16.
16. Hoving-Bolink, A.H., et al., *Perspective of NIRS measurements early post mortem for prediction of pork quality.* Meat Sci, 2005. **69**(3): p. 417-23.
17. Prieto, N., et al., *Application of near infrared reflectance spectroscopy to predict meat and meat products quality: A review.* Meat Science, 2009. **83**(2): p. 175-186.
18. Savenije, B., et al., *Prediction of pork quality using visible/near-infrared reflectance spectroscopy.* Meat Sci, 2006. **73**(1): p. 181-4.
19. Cozzolino, D., et al., *Study of dissected lamb muscles by visible and near infrared reflectance spectroscopy for composition assessment.* Anim. Sci., 2000. **70** (417-423).
20. Andres, S., et al., *The use of visible and near infrared reflectance spectroscopy to predict beef M. longissimus thoracis et lumborum quality attributes.* Meat Sci, 2008. **78**(3): p. 217-24.
21. Troy, D.J. and J.P. Kerry, *Consumer perception and the role of science in the meat industry.* Meat Sci, 2010. **86**(1): p. 214-26.
22. Faustman, C. and R.G. Cassens, *The biochemical basis for discoloration in fresh meat: A review.* Journal of Muscle Foods, 1990. **1**: p. 217-243.

23. Hopkins, D., *Assessment of lamb meat colour.* Meat Focus International, 1996. **5**(11): p. 400-401.
24. Leroy, B., et al., *Prediction of technological and organoleptic properties of beef longissimus thoracis from near-infrared reflectance and transmission spectra.* Meat Science, 2003. **66**: p. 45–54.
25. Edney, M.J., et al., *Analysis of feed barley by near infrared reflectance technology*. Vol. 2. 1994. 33.
26. Williams, P. and D. Sobering, *Comparison of Commercial Near Infrared Transmittance and Reflectance Instruments for Analysis of Whole Grains and Seeds.* Journal of Near Infrared Spectroscopy, 1993. **1**: p. 25-32.
27. Sinnaeve, G., et al., *The Use of near Infrared Spectroscopy for the Analysis of Fresh Grass Silage* 1994. **2**(2): p. 79-84.
28. Williams, P.C., *Implementation of near-infrared technology*, in *Near-infrared technology in the agricultural and food industries*, P.C. Williams and K. Norris, Editors. 2001, American Association of Cereal Chemists: St Paul, Minnesota, USA. p. 143.
29. Hopkins, D., et al., *Relationship between animal age, intramuscular fat, cooking loss, pH, shear force and eating quality of aged meat from sheep.* Australian Journal of Experimental Agriculture, 2006. **46**(7): p. 879-884.
30. Pannier, L., et al., *Associations of sire estimated breeding values and objective meat quality measurements with sensory scores in Australian lamb.* Meat Science, 2014. **96**(2, Part B): p. 1076-1087.
31. Pearce, K.L., et al., *Water distribution and mobility in meat during the conversion of muscle to meat and ageing and the impacts on fresh meat quality attributes — A review.* Meat Science, 2011. **89**(2): p. 111-124.
32. Shackelford, S.D., T.L. Wheeler, and M. Koohmaraie, *Development of optimal protocol for visible and near-infrared reflectance spectroscopic evaluation of meat quality.* Meat Sci, 2004. **68**: p. 371-381.
33. Hopkins, D.L., et al., *Relationship between animal age, intramuscular fat, cooking loss, pH, shear force and eating quality of aged meat from sheep.* Australian Journal of Experimental Agriculture, 2006. **46**(6-7): p. 879-884.
34. Thompson, J.M., *The effects of marbling on flavour and juiciness scores of cooked beef, after adjusting to a constant tenderness.* Australian Journal of Experimental Agriculture, 2004. **44**(7): p. 645-652.
35. Dikeman, M. *Fat reduction in animals and the effects on palatability and consumer acceptance of meat products*. in *Proceedings of the Reciprocal Meat Conference*. 1987. Chicago.
36. Pleasants, A.B., J.M. Thompson, and D.W. Pethick, *A model relating a function of tenderness, juiciness, flavour and overall liking to the eating quality of sheep meat.* Australian Journal of Experimental Agriculture, 2005. **45**(5): p. 483-489.

9 Acknowledgements

Danish Meat Research Institute – particularly Claus Borggaard

Grain Growers Australia – particularly Ian Wesley

New South Wales Department of Primary Industries – particularly David Hopkins

Meat Standards Australia – particularly John Simmonds and Murray Patrick

JBS in Brooklyn – particularly Mark Inglis

Coles – particularly Dale Pemberton