final report

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Veracity of NIR prediction of carcase traits for sheep and beef

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1 Executive Summary

MLA has previously researched (SNGP016D ,PRTC031 and phase 1 of AMQT0051 projects) 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 support 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 second phase of the AMQT0051 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 accreditation by MSA and/or AusMeat.

Interim results from the AMQT0051project report encouraging VISNIR validation models for predicting muscle glycogen level 30 minute post slaughter, loin pHu , loin intramuscular fat percentage and loin tenderness (5 day shear force) from muscle spectra acquired post rigor at 24 hours post slaughter with 83%, 94%, 88% and 98% of lamb carcasses correctly classified at being above or below nominated cut off values of 40umol/g, 5.70, 3.5% and 50 N respectively for the LL muscle. Similiar classification accuracies are reported for the SN muscle. The VISNIR colour .calibration model was 78% for a nominated Hunter lab L cut off value of 37.8. Further analysis of the colour data is required. The results for comparable VISNIR models from pre rigor spectra collected at 30 minutes post slaughter is still being analysed. On the basis of the encouraging.post rigor results 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 crossection of commercial lambs and plants. Before NIR 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.

2 Project 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 technologies where cost effective and accurate, industry is still largely reliant on a mix of objective and subjective assessments to measure and manage these processes.

MLA has previously fund research (SNGP016D, PRTC031, MLA/ NZAgResearch & AMQT0051 projects) to assess the ability of VISNIR spectroscopy (using visible and near

infrared wavelengths) to objectively measure selected beef & sheep meat quality traits including ultimate pH (pHu), ossification (lamb/hogget and mutton), intramuscular fat (IMF) percentage and meat colour to support MSA grading of beef and sheep meat 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. Encouraging results from the initial pilot phase of the AMQT0051 project indicated that NIR technology had the potential to objectively measure a number of meat quality traits that could allow MSA grading of lamb to progress from the current practice of batch grading on pHu alone to individual carcass grading on an expanded suite of traits known to influence lamb eating quality. Substantial enhancements were made to the project methodology used in the initial AMQT0051 pilot study to enhance the likelihood of achieving the stated project objectives. If successful, NIR technology offers the potential to measure these traits on either hot or cold carcasses in real time enabling the technology to integrate seamlessly with modern day industrial meat processing and MSA meat grading practises.

The AMQT0070 final report provides a summary of the key results reported from the AMQT0051 study with regard to with regard to the statistical significance of the VISNIR calibration and/or validation models develop to predict the ultimate pH, meat colour, tenderness (5 day shear force) and intramuscular fat percentage of lamb carcasses both pre- and post-rigor (i.e. 30 mins and 24 hours post-slaughter). The AMQT0070 report also discusses the interpretation of these results and the commericlal opportunities they provide for enhancing MSA grading of lamb.

3 Objectives & Key Milestones

3.1 Project Objectives

- In consultation with MLA, continue the co-ordination and implementation plans for MLA's Adoption Strategy for NIR
- In consultation with MLA continue to develop targeted beef and/or sheep meat R&D projects that further improve the accuracy of NIR technology to support and enhance the objective grading of MSA beef and lamb.
- In consultation with MLA facilitate and brief key beef and sheep meat industry groups that are likely to benefit from the implementating NIR technology within their supply chain. Identify early adopter companies that may be willing to participate in MDC partnership project(s) with MLA on various commercial applications.

- Facilitate (a focused working party consisting of MLA, technical advisors, etc) at least six (6) processor demonstrations in Australia, run preliminary costing model to determine applications and corresponding value propositions.
- Update generic presentation & brochure materials for NIR technology using agreed industry adoption plans and any new technical data derived from new Beef & Sheep R&D projects.
- Facilitate early consultation with standards bodies (Ausmeat), industry bodies (AMPC, AMIC) and commercialiser (ASD)
- Facilitate NIR committee meeting/s
- Report to MLA and recommend next steps in the future commercialisation strategies

3.2 Project Milestones

Achievement Criteria		Due Date
1	Upfront payment upon contract execution and project start	Upon contract execution
2	Liaise and contribute to the final design and experimental plan and processing plant nominated for Phase 2 developed with DEPI and submitted to MLA. • This includes the commissioning and testing of the customised Delta Optics probe and objective data used to formulate the recommended scanning protocols to be implemented in the phase 2 study including the optimum number of probe insertions for carcass sampling.	15-Oct-2014
3	Liaise and support DEPI on site at the nominated processing plant to ensure the accurate collection of all VISNIR muscle spectral data and muscle biopsy samples required for reference measurement assays specified in the AMQT0051 phase 2 contract. Ongoing liaison with DEPI to ensure MLA are updated on the successful completion of the data collection phase. This report will contain the following information • Summary tables of data collected of VISNIR measurements on 200 carcasses. • Summary tables of all on-line carcass measurements and muscle samples collected for reference measurement assays.	30-Dec-2014
4	Liaise and contribute to DEPI's Final report - Phase 2: The final report will provide MLA with an objective statistical and chemometrics analysis of the accuracy of VISNIR to measure pHu, meat colour, intramuscular fat percentage and objective tenderness in pre and post rigor lamb carcasses. The report will provide MLA with recommendations on the potential of VISNIR technology to objectively support MSA grading of lamb.	Amended to 30-May-2015

Achievement Criteria		Due Date
5	Contribute to a draft journal paper prepared for publication if requested.	30-May-2015
6	In collaboration with MLA & DEPI co-ordinate and conduct a workshop with nominated Lamb Supply Chain Group(s) and the MSA sheep pathways committee to brief them on the results and recommendations from the Phase 2 AMQT0051 project. Assuming the results are favourable discuss and identify options for a full commercialisation plan for VISNIR technology to support the MSA grading of lamb including grading on ultimate pH, meat colour, tenderness (shear force) and intra-muscular fat percentage.	Amended to 30-Jun-2015

4 Lamb NIR project results

The results summarised in Tables 1 & 2 below are derived from the AMQT0051 final report submitted by DEDJTR (previously known as DEPI) the contracted research agency. Milestones 1 to 3 have been successfuly achieved. Milestone 4 is 80% complete.due to delays in processing some of the reference standards due to breakdowns in key laboratory equipment, the size and complexity the data base requiring analysis and time constraints on the specialist external collaborators contracted to conduct the chemometric analysis.MLA project management have been kept informed about these dealys. DEDJTR have prepared a draft journal paper to complete milestone 5 and assistance is being provided to DEDJTR to prepare a power point presentation for DEDJTR to present the project results to a lamb industry supply chain meeting in mid July nominated by MLA. MLA (Alex Ball) has indicated Baud & Assoc Pty Ltd is not required to attend this industry consultation meeting. It is expected all milestones can be completed in July 2015. A number of the interim results are supportive of progressing the project to a third and final phase to further validate the accuracy of the most promising VISNIR prediction models with commercial industry.

Table 1 Post rigour VISNIR validation models for predicting muscle glycogen level, muscle pHu, meat colour, intra muscular fat percentage and tenderness (5 day shear force) from spectra collected on chilled lamb carcasses 24 hours post slaughter using the PAS probe couple with the Terra Spec spectrometer

VISNIR Model	Reference /Validation model statisitics	Glygogen 30 minutes post slaughter	pHu (direct)		Meat colour (Hunter lab L value)		Intramuscular fat %		Tenderness (5 day shear force)	
Muscle		LL	LL	SM	LL	SM	LL	SM	LL	SM
Calibration	Ref N	202	203	203	178	178	203	203	203	203
	Ref mean	61.71	5.63	5.64	34.14	32.16	3.3%	3.1%	34.2N	51.5N

	Ref SE									
	Calibration N spectra	262	262	296			262	296	262	296
	Calibration N samples	131	131	148	178		131	148	131	148
	Calibration model SECV	11.9	0.1	0.1	3.77	Na	0.85	0.75	8.5	10.1
	Calibration model R^2	0.03	0.22	0.37	0.60	Na	0.36	0.21	0.1	0.12
	No. of PLS factors	5	5	7	3		6	7	3	4
	Nominated cut off value				37.8					
	Model classification accuracy ie % correctly assigned				78%					
Validation	Validation N	48	49	48			48	48	49	48
	Validation model SEP	11.6	0.2	0.2			0.6	0.8	7.2	10.0
	Validation model R^2	0.39	0.39	0.46			0.38	0.29	0.13	0.12
	Nominated cut off value	40umol/g	5.7	5.7			3.5%	3.5%	50N	50N
	Model classification accuracy ie % correctly assigned	83%	94%	96%			88%	77%	98%	70%

Table 2 Pre rigour VISNIR validation models for predicting muscle pHu, meat colour, intra muscular fat percentage and tenderness (5 day shear force) from spectra collected on chilled lamb carcasses 30 minutes post slaughter using the pencil probe coupled with the Terra-Spec spectrometer

VISNIR Model	Reference /Validation model statisitics	pHu (indirect)		Meat colour (Hunter lab L value)		Intramuscular fat %		Tenderness (5 day shear force)	
Muscle		LLGlycogen	LLpHu	LL	SM	LL	SM	LL	SM
Calibration	Ref N	203	203	178	178	203	203	203	203
	Ref mean	5.63	5.64	34.14	32.16	3.3%	3.1%	34.2N	51.5N
	Ref SE								
	Calibration N			178					
	Calibration			1.67					

	model SECV								
	Calibration model R^2			0.4					
	No. PLS factors			3					
	Nominated cut off value			34					
	Model classification accuracy ie % correctly assigned			71					
Validation	Validation N								
	Validation model SEP								
	Validation model R^2								
	Nominated cut off value								
	Model classification accuracy ie % correctly assigned								

5 Discussion

The predictive accuracy of the VISNIR validation models presented in Tables 1 & 2 for each of the meat quality trait assessed needs to be considered against several different statistical criteria rather than be assessed on a single statistical criteria alone for the reasons discussed below:

R^2 values- are as measure of the accuracy of NIR models prediction models but are dependent on factors such as the variation ie range and standard deviation that exists in the in the reference standards. They are also dependent on whether the NIR prediction is based on a direct or indirect measure of the reference standard it is being calibrated to. Whilst the project endeavoured to maximise the variation in the traits assessed when selecting the lamb carcasses for scanning in most instances the majority of the reference samples are bunched around the mean of a normal distribution curve. Secondly the NIR prediction models for a number of the traits assessed (pHu, & shear force) are an indirect measure & not a direct measure of the reference standard used for calibration purposes. In such cases NIR can't directly measure the chemical bonds that largely control the expression of that

trait but indirectly measures that trait by measuring changes to chemical bonds associated with that trait. Under such circumstances NIR prediction models are likely to be less accurate (lower R^2). For example NIR uses a quite different methodology to measure pH compared to a conventional pH meter. Conventional pH meters measure proton (H+) concentrations. NIR is unable to measure H+ levels directly but rather measures pH indirectly be measuring stretching/distortion in the OH bands. NIR will react to changes in protein structure and how translucent the meat is (both being pH dependent). When we go from pH = 7 down to pH =6 the proton concentration changes from 0.0000001 mol/liter (10 to the minus 7th)to 0.000001 mol/liter (10 to the minus 6th). NIR can't measure anything that low. But the indirect measurement may be OK!

Comparison of the Reference standard standard error (Ref SE) to the VISNIR validation model standard error (SECV). Whilst the reference standard is treated as the "gold" standard it too is subject to measurement error. Consequenlty the predictive accuracy of the VISNIR may be acceptable if the measurement errors in the reference standard and VISNIR predicted value are comparable. For example other pH related studies have shown that pH cannot be determined with a pH meter and glass electrode with a reproducibility better than 0.07 units (67% confidence level, two different instruments, two different operators). This makes our current VISNIR SECV estimate of 0.1 look respectable.

Number of PLS factors used in the model. The number of PLS factors used in the VISNIR validation model provides an indication of the reliability of the model. Generally the higher the number of PLS factors the less reliable the model.

VISNIR model classification accuracy around nominated cut off values: The ultimate test of VISNIR technologies value to commercial industry as a grading tool is its ability to correctly classify above or below a cut off value nominated by industry for the meat quality trait measured. The target is to achieve a 90% plus classification accuracy.

Generally the calibration model statistics for most models are quite poor. However because commercial industry are not likely to require the absolute figure but rather whether it is above or below a nominated cut off value then we can get quite a good prediction. This is helped by averaging the results of the two spectral predictions. Based on the interim results available to date number of positive results were obtained from this study that offer the potential for significant enhancements to current MSA lamb grading practices.

1. **VISNIR prediction of pHu** . Current MSA practice is to measure a subset of carcases within a consignment for pHu with extrapolation to the cohort. Based on the

SECV estimates and classification accuracies of the VISNIR models developed the results of this study suggest VISNIR could be used to measure pHu in all carcasses at 24 hours post slaughter directly and classify them into high or low pHu categories (based on a nominated cut off value). This would provide a more thorough appraisal of pHu across a consignment providing a more reliable grading outcome. If required only high pHu carcases would need to be retested with a pH meter to verify classification status with falsely classified carcases returned to the appropriate pH cohort..

The results of a second alternative approach using muscle glycogen values at 30min post slaughter to predict pHu and similarly classify carcases as into high or low pH categories are not yet complete. The VISNIR model to predict muscle glycogen levels at 30 minutes from scans collected at 24 hours post slaughter was 83% accurate in classifying carcasses above or below a nominated cut off value of 40umol/g. If positive results are obtained to predicting pHu from the level of pre rigor muscle glycolytic metabolites then this application would give processors the option to grade carcasses for pHu “hot” off the kill floor rather than the current requirement of waiting until the carcasses have reached rigor.

Also the two independant pHu VISNIR prediction models could be used in tandem to predict pHu. One estimate could be derived from the 24 hour post slaughter direct prediction of pHu. A second estimate based on muscle glycolytic metabolites could be predicted and the two compared. Using two estimates derived from 2 different reference standards may further improve the predictive accuracy of VISNIR to classify carcasses into high and low pH categories. In theory muscle glycolytic potential (GP is the sum of all glycolytic metabolites) remains constant from slaughter to rigour. This allows muscle glycolytic metabolite levels to be reliably calculated at any nominated standard time point ie from slaughter to 24 hours post slaughter .

2. **VISNIR prediction of tenderness (5 day shear force) and intramuscular fat percentage.** 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 IM fat % 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.

3. **VISNIR prediction of meat colour** The SECV and R^2 estimates calculated for the VISNIR calibration models for meat colour provided in Tables 1 & 2 has improved our understanding of the factors influencing VISNIR's ability to measure meat colour but further improvement in the model accuracies is required before NIR could be used as a reliable grading tool to support MSA grading of lamb for meat colour. The results highlight the technical challenges associated with objectively measuring colour. As reported in the comparable NIR beef study (AMQT0052) a strategy of basing NIR calibration /validation models exclusively on MSA grader assessment colour scores that are subjectively based as the sole reference standard was unsuccessful. The results from the AMQT0051 study reports a similiar outcome for lamb. The AMQT0051 report also highlights the relatively low correlation that exists even between the 3 objective colour instruments used in the study to objectively measure colour. This is possibly due to differences in lighting conditions and angle of detection which vary between all of the colorimeter instruments used in this study. Further analysis of the colour data set is recommended to identify the best option(s) to further develop an appropriate reference standard for measuring meat colour in lamb

6 Recommendations

Whilst the results are not yet complete for this project the interim results support the project progressing to a third and final validation/commercialisation phase as per the original project proposal. It is recommended that:

1. **Validation of the VISNIR prediction models** reported for muscle glycogen, pHu, intramuscular fat percentage and tenderness (5 day shear force) from post rigor scan data collected at 24 hours post slaughter should be further tested on a wider cross section of commercial industry and /or Sheep CRC lambs processed at 2 different plants. Validation of the comparable VISNIR prediction models reported for pre rigor scan data collected at 30 minutes post slaughter could also be considered for inclusion subject to the predictive accuracy of the models being comparable to the post rigor 24 hour models when their results become available.
2. **Additional analysis of the meat colour data** be conducted to further investigate options for objectively measuring colour in lamb before inclusion in recommendation

1 above. The AMQT0051 database on objective lamb meat colour measurements provides a valuable resource (3 objective & 1 subjective measures of lamb meat colour either bloomed and unbloomed) to further explore other model or instrument options to predict meat colour in lamb. Whilst the analysis completed to date meets the requirements of the AMQT0051 contract consideration needs to be given to additional analyses that may improve the VISNIR prediction model accuracy for meat colour to the 90% plus levels required.

3. **Instrumentation/software issues** Before commercial industry could implement NIR technology to support MSA grading of lamb it will require some serious investment to upgrade and industrialise the NIR spectrometer, probes and supporting software that underpin the technology. The instrumentation & software currently used requires a high level of operator skill to ensure the spectra acquired is of high quality, calibration drift is minimised and the spectra acquired is successfully downloaded & processed. All these tasks can be simplified but it will require additional investment before the technology is suitable for implementation by industry.
4. **NIR technology to support beef MSA grading –** The comparable Beef NIR project (AMQT0052) was put on hold until the results from the current lamb NIR project were known. Whilst it is still premature to make a decision the methodology used in the current AMQT0051 project is proving to be more successful than the methodology used in the beef NIR project. Subject to the final results of the lamb NIR project being known they may also be equally applicable to support beef MSA grading.