final report

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Prepared by: Baud & Associates Pty Ltd

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Locked Bag 991
NORTH SYDNEY NSW 2059

Veracity of NIR prediction of carcass traits

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1 Executive Summary

MLA has previously researched (SNGP016D ,PRTC031 and joint MLA/ NZAgResearch projects) the ability of VISNIR spectroscopy (using visible and near infrared wavelengths) to objectively measure selected beef & sheep meat quality traits including ultimate pH , ossification, 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. This project reports on two additional studies commissioned by MLA to further refine the VISNIR meat quality prediction models nominated by MSA with the objective of achieving the grading accuracies required for the technology to achieve accreditation by MSA and/or AusMeat.

The beef NIR project (AMQT0052) reported that the VISNIR validation models for pHu, MSA meat colour, MSA marble score and ossification score all required further improvement in their accuracies before NIR could be used as a reliable grading tool to support MSA grading of beef. It is recommended MLA not progress further with the beef NIR project until the sheep NIR project (AMQT0051) is completed given it will provide clarification on recommendations suggested for the next phase of the beef project to achieve the VISNIR grading model accuracies required. The sheep NIR project reported that the VISNIR validation model for pHu was very encouraging. Using pHu values predicted from VISNIR glycogen estimates at 30 min post-slaughter and setting a classification cut-off at pH 5.75, 85% of samples were classified into the correcty category based on comparison of predicted vs actual pHu. The results suggest this alternate approach to measuring pH using VISNIR may be a superior option to the current MSA practice of measuring a subset of carcases within a consignment for pHu with extrapolation to the cohort.

As reported in the beef NIR project the VISNIR validation models reported for those MSA meat quality traits assessed on a categorical grading scale system i.e. meat colour, and

marbling values lacked sufficient accuracy to be able to reliably grade lamb carcasses on these traits. It is recommeded that the predictive capability of VISNIR be re-evaluated using alternative objective reference measures of these meat quality traits using new objective measurement technology known as the VideometerLab. Based on the results reported it is recommended that the Sheep NIR project progress to the next phase incorporating the recommendations to achieve the VISNIR model accuracies for pHu, meat colour, and intramuscular fat percentage required to reliably support the accurate grading of MSA lamb.

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 objective measurement technologies are available, industry is still largely reliant on a mix of objective and subjective assessments to measure and manage these processes. Despite a concerted effort by the research community over the past decade, 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 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 (SNGP016D, PRTC031 and joint MLA/ NZAgResearch projects) 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. 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 and/or AusMeat could be achieved.

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

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
- Identify early adopter companies that may be willing to participate in MDC partnership project(s) with MLA on various commercial applications
- Prepare generic presentation & brochure materials for NIR technology using existing adoption plans and technical data derived from the Beef and Sheep R&D work
- 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
- Facilitate early consultation with standards bodies (Austmeat), 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	Signing of the contract	30-Sep-2011
2	NIR Beef R &D project AMQT0052 completed (at 1 processing company) with data captured from NIR equipment and MSA conventional meat quality measurement for comparison and model development	15-Feb-2012
3	Correlation studies and Chemometrics analysis of beef data completed. AMQT0052 milestone report provided to MLA on NIR calibration model development to predict nominated MSA meat quality measurements with equal or superior accuracy to conventional measurement methods	25-Mar-2012

Achievement Criteria		Due Date
4	NIR Sheep R &D AMQT0051 project completed (at 1 processing company) with data captured from NIR equipment and MSA conventional meat quality measurement for comparison and model development	15-Mar-2012
5	Correlation studies and Chemometrics analysis of sheep data completed. AMQT0051 milestone report provided to MLA on NIR calibration model development to predict ultimate pH, meat colour & age category when measured pre rigor on the hot carcasses with equal or superior accuracy to conventional measurement methods.	25-Apr-2012
6	Consultations with Ausmeat to gain agreement of nominated Meat quality traits measured using NIR technology completed	15-May-2012
7	Validation studies to attain Aus meat endorsement of nominated Meat quality traits measured using NIR technology completed	30-Jun-2012
8	Industry consultation to inform beef & lamb processors on the capabilities of NIR technology to measure key MSA meat quality traits and the potential commercial value of the technology to the processing sector. Meeting to seek industry recommendations on facilitated adoption of NIR technology to measure nominated MSA MQ traits, implementation steps (early adopters & applications) based on industry consultation; report recommendations to MLA.	15-Aug-2012

4 Beef NIR project results

The results summarised in Table 1 below were derived from the AMQT0052 milestone 3 report submitted by the NZAgResearch the contracted research agency. The recommendations were developed by NZAgResearch in consultation with the project co- ordinator Stuart Baud.

Table 1 VISNIR calibration and validation models for meat colour, pHu, ossification and MSA marble score in beef carcasses

Model		Meat colour	pHu	Oss	MSA Marble score
		(MSA colour		100-590	
Calibration	n	334	299	217	370
	SEC	0.81	0.11	38	71
	R^2	0.67	0.75	0.91	0.85
Validation	n	271	297	145	371
	SEC	0.86	0.12	31	84
	R^2	0.60	0.73	0.78	0.79

The SEC/SECV and R² estimates calculated for the VISNIR calibration and validation models detailed above indicate that further improvement is still required in the model accuracies for measuring all four meat quality traits before NIR could be used as a reliable grading tool to support MSA grading of beef.

Given these results MLA decided not to progress the AMQT 0052 project beyond milestone 3 at this stage until the complete results from the sheep NIR project were available.

Discussion

A number of fundamental issues need to be considered before there is any continuation in the proposed AMQT0052 R&D program.In particular

1. **Meat colour and marble score model options**. The current strategy of basing all NIR calibration /validation models exclusively on MSA grader assessment scores that are subjectively based as the sole reference standard needs to be re- considered. It is suggested that more success may be achieved if the calibration and validation models for meat (& fat) colour and marble score if they were assessed against reference standards obtained using new objective measurement technology known as the VideometerLab. This instrument provides an objective

measure of colour and intramuscular fat under standardised lighting conditions in terms of both spectral absorbance values at defined wavelengths and RGB pixel colour values the latter aligning most closely to colour as perceived by the human eye http://www.youtube.com/watch?v=syzJihVd7Fw or www.videometer.com).
The Videometer Lab could be initially be used provide the objective reference values of meat colour for a range of meat samples scanned. This data could then be used to develop VISNIR regression models required to be able to predict meat (& fat) colour and marble score using VISNIR only (Trinderup et al).

2. **Ossification model options**- The current weakness in the VISNIR calibration/validation model for ossification is directly attributable to the inaccuracy of the current lumbar scan site due to frequent damage from soft siding. This site is used to predict ossification scores in the 160-200 range. Two alternative sites have been shortlisted as possible alternatives. These alternative sites may also enable ossification scores to be predicted on carcasses where the feather bones have been removed on the kill floor. Industry has also expressed an interest in being able to use a comparable "hot" beef carcass ossification model. Project PRTEC031 demonstrated that accurate hot and cold carcass NIR models were developed to differentiate between lamb, hogget and mutton based on rib bone ossification measurements taken on either hot or chilled carcasses.

3. **pHu model options**- 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 by 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! Other pH related studies have shown that pH cannot be determined with a pH meter and glass electrode with reproducibility better than 0.07 units (67% confidence level, two different instruments, two different operators). This makes our current NIR SEV (standard error of validation) estimate of 0.12 look respectable.
Including a 2nd reference standard based on muscle glycolytic metabolites & buffering capacity may further improve the current validation model to the accuracy required. In theory muscle glycolytic potential (GP is the sum of all glycolytic metabolites) remains constant from slaughter to rigour. This allows muscle glycogen levels at any nominated standard time point to be reliably calculated ie at

slaughter. We know from previous studies (MLA project SNGPI016D) muscle glycogen and/or GP is strongly correlated with pHu. Using the results from 2 independent NIR pH prediction calibration/ validation models may achieve the classification accuracies required by MSA & Ausmeat.

5 Lamb NIR project results

The results summarised in Table 2 below were derived from the AMQT0051 milestone 2 report submitted by VicDPI the contracted research agency. The recommendations were developed by VicDPI and other external contributing organisations in consultation with the project co-ordinator Stuart Baud. Milestones 4 &5 were successfuly achieved with the results supportive of progressing the project beyond the pilot phase.

Table 2 VISNIR validation models for predicting muscle glycogen, pHu, MSA meat colour (beef chips) and MSA marble score from spectra collected on hot lamb carcasses (30 minutes post slaughter) using 2 different spectrometer/probe systems

Model		Meat colour		pHu	Muscle Glycogen	MSA Marble score
		Hunter lab L	(MSA beef colour chips)			
Terra spec/PAS	n	208	217	210	197	107
	SEC	2.91	0.81	0.18	8.52	75
	R^2	0.72	0.85	0.53	0.72	0.54
ASD 1800/ ASD bifurcated pencil	n	204	212	196	203	106
	SEC	2.6	0.88	0.19	9.98	79
	R^2	0.77	0.82	0.49	0.64	0.46

VISNIR was able to provide reasonable prediction models for muscle glycogen (r^2 0.64 to 0.72, with SEP 8.5 to 9.9; range is 0 to 75 mg/100g). Prediction models for Hunter L were also good, with r^2 values from 0.72 to 0.76 with SEP 2.6 to 2.9 (range is 20 to 50 units). Prediction models for pHu were weaker than for direct biochemical or biophysical traits (r^2 1.49 to 0.53; with SEP of 0.18 to 0.19; range 5.35 to 6.7). Based on these accuracies, it is considered not appropriate to use VISNIR at 30min post slaughter to predict pHu directly. Due to the nature of the data collected for Marbling and colour grading (categorical data), VISNIR models were not able to provide good discrimination of samples within muscle type for these parameters.

The VISNIR predicted values for glycogen at 30 min post slaughter were used within a statistical model developed from actual glycogen values that accounted for kill day in prediction of pHu. Using this 2-step process to predict muscle pHu in the LT, our model accounted for 45% of the variation in the data with an SEP of approximately 0.15 across the modelled range of 30 to 80 mg/100g glycogen.

Using pHu values predicted from VISNIR glycogen estimates at 30 min post-slaughter and setting a classification cut-off at pH 5.75, 15% of samples were classified into the wrong category based on comparison of predicted vs actual pHu. If we consider the limitations of this study, which include a) using a VISNIR spectra at 30 minutes post-slaughter taken at a site distinct from where pHu and glycogen samples were taken, b) approximately 23 hours of time difference between spectra acquisition and pHu attainment, and c) measurement of pHu in carcases is subject to variation in the order of 0.1 pH units, then a 15% failure rate in classification at pH 5.75 is not a bad result. Using the model developed here, the majority of incorrectly classified carcases were low pHu carcases classified as having pHu greater than 5.75.

Discussion

A number of positive results were obtained from this pilot study that offer the potential for significant enhancements to current MSA lamb grading practices.

1. NIR prediction of pHu. Current MSA practice is to measure a subset of carcases within a consignment for pHu with extrapolation to the cohort. The results of this study suggest an alternate approach using VISNIR to predict muscle glycogen values at 30min post slaughter to predict pHu and classify carcases as into high or low pH categories (based on cut-off of pH 5.75) to provide a more thorough appraisal of pHu across a consignment. 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. Based on this outcome and approach we suggest that this project proceeds to the next phase of evaluation and testing. This evaluation should compare the relative accuracy of assigning carcases to MSA pHu specifications using VISNIR predicted glycogen classification as opposed to the relative accuracy of the current MSA practice. If successful 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.

2. NIR prediction of meat colour and MSA marble score. The VISNIR validation models reported for those MSA meat quality traits assessed on a categorical grading scale system i.e. meat colour, and marbling values lacked sufficient accuracy to be able to reliably grade lamb carcasses on these traits. It is recommeded that the predictive capability of VISNIR be re-evaluated using alternative objective reference measures of these meat quality traits. For reasons already outlined for the beef project under 4.2.2. it is recommended that the

calibration and validation models for meat colour (& if required fat colour) and marble score be assessed against reference standards obtained using new objective measurement technology known as the VideometerLab

3. Spectometer/ probe selection. Two different spectrometer/probe configurations were tested and reported on in the AMQT0051 pilot study. Surprisingly despite there being major differences in the physical size of the 2 probes there was only relatively minor differences in the accuracies of the VISNIR validation models reported for all traits. However there are major differences in commercial acceptability of the 2 probes tested from a processors perspective. The ASD bifurcated pencil probe is much smaller which enables the loin muscle to be scanned relatively non-invasively without any potential negative carcass downgrading impact. The larger PAS probe requires a cut surface of the loin muscle to be exposed hence its commercial acceptability is more likely to be limited to a boning room environment. Given the non homegenity of loin muscle with regard to meat colour, pHu and intramuscular fat distribution it is recommended design options be investigated for the ASD bifurcated pencil probe that would at least double the surface area of muscle tissue scanned without any detrimental downgrading impacts on the carcass.

6 Recommendations

On the basis of the results reported, it is recommended that the Sheep NIR project progress to the next phase incorporating the recommendations to achieve the VISNIR model accuracies for pHu, meat colour, and intramuscular fat percentage required to reliably support the accurate grading of MSA lamb. The next phase of the Sheep NIR project should:

- Compare the relative accuracy of assigning carcases to MSA pHu specifications using VISNIR predicted glycogen classification as opposed to the relative accuracy of the current MSA practice. If successful 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.
- The predictive capability of VISNIR be re-evaluated using alternative objective reference measures of meat colour and intramuscular fat levels. It is recommended that the calibration and validation models for meat colour and instramuscluar fat percentage be assessed against reference standards obtained using objective measurement technology including the VideometerLab.
- Given the non-homegeneity of loin muscle with regard to meat colour, pHu and intramuscular fat distribution it is recommended that design options be investigated for the ASD bifurcated pencil probe that would at least double the surface area of muscle tissue scanned without any detrimental downgrading impacts on the carcass.

It is recommended MLA not progress further with the beef NIR project until the sheep NIR project (AMQT0051) is completed given that it will provide clarification on recommendations suggested for the next phase of the beef project to achieve the VISNIR grading model accuracies required.