

# final report

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## **Feasibility of measuring ossification by NIR**

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## 1 Introduction

One of the key quality parameters for Australian MLA graded beef is ossification, or degree of bone development.

This is largely an age issue, but feeding regimes, genetics and other environmental factors impact upon it also. While one could imagine each animal may have a known age, in practice this is not a good link, and even were it so, the other factors mean same-age animals may have widely different ossification levels.

MLA has shown ossification has been shown to have a significant impact on eating quality. Previous work on sheep [0] has shown a potential ability for NIR to measure ossification. The measurement point showing most promise was upon the 6<sup>th</sup> rib, within the chest cavity. However, it was not known if this would hold for cattle, or indeed a new population. This trial looked to identify the best measurement location, and obtain some rough estimates of likely precision that may be possible.

## 2 Outcomes

NIR is likely to be able to measure ossification to around at least +/- 50 units. A more focused trial to establish the specific operation and measurement parameters may well improve on this estimate.

The previously found best site (6<sup>th</sup> Rib) did not show as the best measurement site. The best measurement site overall was probably the T6C site, measured with the DPI instrument, although the G1 site measured with KES was also reasonable. The larger data set available for the KES result (several hundred carcasses) gives more confidence in the outcome for that measurement point.

## 3 Equipment

Three spectrometers were available to test together to identify the feasibility of ossification, designated as DPI, USA, KES (see Table 1 for details). All units cover both visible and NIR wavelength ranges, and were relatively mobile and suitable for meat plant operation.

The USA device had a particularly large measurement head, and this became a major issue with this head physically unable to reach the 6<sup>th</sup> Rib measurement location. Further, the weight of the head and difficulty moving from site to site meant a minimal amount of carcasses were able to be processed on this unit.

The DPI unit was slow to capture spectra, and unable to reconcile scanning the barcode directly to obtain a sample name. This impacted considerably upon the carcasses and sites within a carcass that could be scanned.

The KES unit captured in around 1 second or less, and was backpack mounted, allowing considerable versatility and movement. Coupled with the barcode input of sample name meant considerable spectra were obtainable at line speeds.

## 4 Trial details

### 4.1 Location

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Melbourne based meat processors, Swifts, kindly offered their facility and access to commercial slaughtered carcasses for the trial. While one always tried to minimize the impact on production, no trial can be truly zero-impact, and Swifts assistance and forbearance is gratefully acknowledged.

A suitable region of the chain was identified and we were able to process a number of carcasses, while also venturing into various cool rooms to obtain specific desirable carcasses.

### 4.2 Property matrix

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The most desirable matrix would have been to obtain reasonably even carcass numbers in each of the ossification ranges (Table 3). However, with various operational issues and equipment limitations, we were only able to obtain the numbers shown. Not all locations were available for every carcass.

## 5 Data processing

Considerable data processing was required to align the data sets and link the spectra to the ossification scores (through the recorded carcass number). There remained a number of ambiguous data points, where the same carcass number occurred on separate days or kill groups. Where these could not be reconciled, there was little option but to omit both occurrences. ASD spectral data was processed by Matt Kerr of DPI, and provided later. A complete kill group (group 4) was not provided. Unfortunately this included the bulk of the in-line scans for the instrument.

USA spectral data was the same format as the DPI, as both are from the same manufacturer. These units were internally corrected for drift and whatnot, and subsequent data processing needed only take the spectra as provided.

KES spectra comprised the individual sample, reference and tile spectra. Data processing consisted of the usual ratio between sample and reference, then ratio this against the calibration tile (also treated as ratio Sample/Reference). Spectra were inspected and some clear outliers identified and omitted. There were few such spectra for any of the units.

Collation to the Ossification (and other) plant data proved somewhat problematic, as the records for some carcasses were ambiguous. Where these could not be resolved reasonably, these spectra were dropped from the data analysis.

## 6 PLS analysis

For simplicity in the tables and graphs, acronyms are used wherever possible. These are explained in Table 2. Reported here are only those preprocessing regimes that resulted in an at least reasonable fit between the predicted and measured outcomes.

A great many other preprocessing options were also tried with many data sets, before finally arriving at the one reported here. In particular, the normally useful SNV (Standard normal Variate) and Mean-Centred (or Autoscaled) were very poor at fitting for all data sets.

In every case investigated, “GLS + AS” (see Table 2) was significantly better fitting than any other combination of pre-processing attempted.

There were a number of measurement sites with spectra measured. However many of these were quite quickly dropped off once the operational issues were identified. The breakdown of sites and carcasses is shown in Table 4. Only those cases where around 30 or so different carcasses were measured (or more) were included for analysis. These are noted in Table 4.

## 6.1 PLS Processing

As discussed above, in every case, the same pre-processing (GLS+AS) was employed. Furthermore, the analysis was by cross-validation. Even for those cases with sufficient samples to justify using more formal Calibration/Validation sets, it was felt this would not allow a direct comparison between other cases evaluated differently. In these cases, while the calibration fit (RMSEC) is noteworthy, the best estimate of how the calibration would perform on new samples is provided by the cross-validation metric (RMSECV).

In the absence of a test data set, a good measure of the degree of “over-fitted” is to observe how close the calibration and cross-validation (RMSEC and RMSECV). Note for example the “Air” case for DPI. This was accidentally left in the analysis system (analyzing blind). Clearly the prediction for air-shot from the DPI is somewhat suspect. Note the extreme poor fit by cross-validation. This is telling us there is sufficient randomness to “fit” a small number of samples anyway, but that calibration is just fitting the specific noise, and a new sample will be completely non-predicted. Hence the RMSECV around 500.

The best fits for the three units are extracted and shown in Table 6, along with the correlation fit ( $R^2$ ). Also, for comparison, the 6<sup>th</sup>-Rib site is left for DPI. The plots of the calibration fit for these are in Figure 1 through Figure 4.

One needs be careful interpreting the RMSEC fit values given the excellent fit for Air!

However, looking at the graphs (Figure 1 through Figure 4), its clear there is a quite reasonable predictability for NIR over the complete range. The 70-unit precision is probably too high for commercial acceptance, however

- All three units have targeted “something” related to ossification
- There is clearly a response here that NIR spectrometers are reacting to
- The cross-validation agreement is reasonable enough (aside from the 6<sup>th</sup> rib) to have some confidence there is a valid correlation.

## 7 Tables

**Table 1: Spectrometers used in the trial**

Unit	Manufacturer	Location	Type	Probe	Spectral range
DPI	ASD Ltd	Melbourne, Australia	Hybrid Monochromator + Diode Array	1cm reflectance	400-1800 nm
KES	KES Analytical Inc	Hamilton, NZ	Diode Array	1.2cm interactance	400-1700 nm
USA	ASD Ltd	Hamilton, NZ	Hybrid Monochromator + Diode Array	2cm reflectance	400-1800 nm

**Table 2: PLS Preprocessing abbreviations**

<b>Acronym</b>	<b>Pre-process</b>	<b>Explanation</b>
<b>SNV</b>	Standard Normal Variate	For every spectrum: subtract mean and divide by the standard deviation. This gives spectra mean zero, with SD = 1.
<b>MSC</b>	Multiplicative Scattering Correction	Regress each spectrum against the spectra mean, retain the fitted spectrum
<b>MC</b>	Mean-Centred	Subtract each spectrum from the overall spectral mean
<b>AS</b>	Autoscale	Subtract each spectrum from the overall mean, and divide by the overall SD for each data point. Similar to SNV, but treats by global mean, rather than individual mean, etc.
<b>GLS</b>	Generalised Least-Squares	Models the data set as a generalized least-squares distribution. Retains the residuals of the fitted spectra. Very useful to remove spurious cross-correlations, but has disadvantage of destroying the “look” of the spectra.

**Table 3: Ossification ranges measured**

<b>Ossification</b>	<b>Carcasses scanned at that ossification level</b>		
	<b>ASD</b>	<b>KES</b>	<b>USA</b>
<b>0-50</b>	0	0	0
<b>50-150</b>	11	74	9
<b>150-250</b>	14	204	14
<b>250-350</b>	3	6	3
<b>350-450</b>	0	2	0
<b>&gt; 450</b>	1	47	1

**Table 4: Carcass measurement points data sets and PLS outcomes**

Unit	Site	Location	Spectra	Bodies	Unique Oss	Vectors	RMSEC	RMSECV	File
KES	1	AI	354	305	16	5	88.9	135.8	PLS_KES_AI_ALL
	2	CC	708	307	16	5	73.3	93.0	PLS_KES_CC_ALL
	3	CT	217	3	3				
	4	G1	97	30	12	1	52.1	75.8	PLS_KES_G1_All
	5	G2	99	31	12	3	38.6	102.9	PLS_KES_G2_All
	6	R0	4	2	2				
	7	R1	4	2	2				
	8	R2	4	2	2				
	9	R3	4	2	2				
	10	R4	4	2	2				
	11	R5	4	2	2				
	12	R6	4	2	2				
	13	R7	4	2	2				
	14	R8	4	2	2				
	15	R9	4	2	2				
	16	RB	99	31	12	5	41.7	154.2	PLS_KES_RB_All
	17	SP	2306	31	12	5	62.4	107.8	PLS_KES_SP_All
	18	UU	30	23	10	2	76.7	768.2	PLS_KES_UU_All
	19	VT	2381	31	12	3	63.1	98.0	PLS_KES_VT_All
DPI	1	Air	321	30	12	4	12.6	471.6	PLS_DPI_Air
	2	L3B	288	30	12	4	12.3	98.6	PLS_DPI_L3B
	3	L3C	288	30	12	4	8.3	84.9	PLS_DPI_L3C
	4	ML	288	30	12	5	8.2	93.2	PLS_DPI_ML
	5	Rib6	288	30	12	5	6.6	67.5	PLS_DPI_Rib6
	6	S1B	288	30	12	6	6.5	75.1	PLS_DPI_S1B
	7	S1C	291	30	12	5	9.9	134.7	PLS_DPI_S1C
	8	T12B	288	30	12	4	8.0	79.3	PLS_DPI_T12B
	9	T12C	288	30	12	5	9.7	78.2	PLS_DPI_T12C
	10	T6B	288	30	12	5	7.0	87.2	PLS_DPI_T6B
	11	T6C	288	30	12	2	26.1	53.0	PLS_DPI_T6C
USA	1	L02	29	2	2				
	2	L03	331	25	12	5	8.7	81.9	PLS_USA_L03
	3	S02	353	27	12	1	37.6	86.5	PLS_USA_S02
	4	T05	13	1	1				
	5	T06	362	26	12	5	9.6	74.5	PLS_USA_T06
	6	T11	12	1	1				
	7	T12	337	26	12	5	8.1	81.8	PLS_USA_T12

**Table 5: Measurement locations explained**

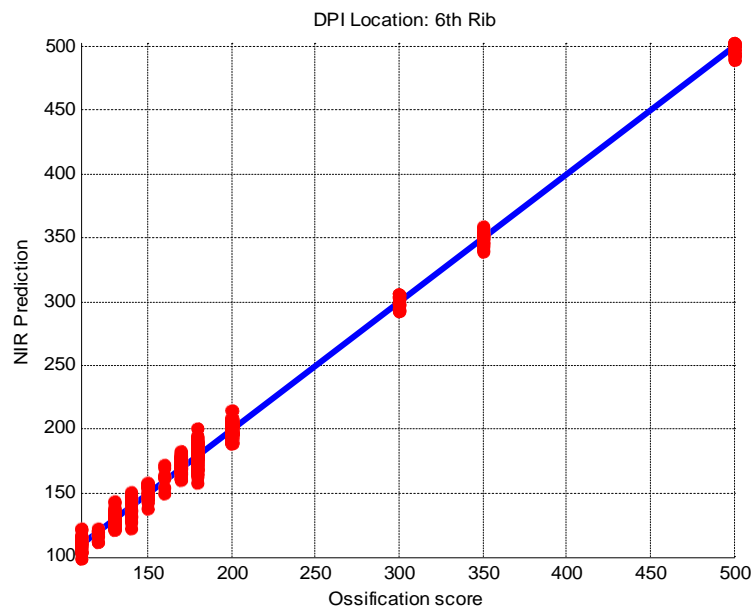
Unit	Site	Location	Explained
<b>KES</b>	1	AI	
	2	CC	
	3	CT	
	4	G1	
	5	G2	
	6	R0	Area before ribs defined
	7	R1	First rib
	8	R2	Second rib
	9	R3	Third rib
	10	R4	Fourth rib
	11	R5	Fifth rib
	12	R6	Sixth rib (same position as DPI)
	13	R7	Seventh rib
	14	R8	Eighth rib
	15	R9	Ninth rib
	16	RB	
	17	SP	Spinus process
	18	UU	
	19	VT	Vertibrae at Sacral
<b>DPI</b>	1	Air	
	2	L3B	
	3	L3C	
	4	ML	
	5	Rib6	
	6	S1B	
	7	S1C	
	8	T12B	
	9	T12C	
	10	T6B	
	11	T6C	
<b>USA</b>	1	L02	
	2	L03	
	3	S02	
	4	T05	
	5	T06	
	6	T11	
	7	T12	



**Table 6: Best PLS predictions for each unit**

Unit	Site	Location	Vectors	RMSEC	RMSECV	R2
KES	4	G1	1	52.1	75.8	
DPI	5	Rib6	5	6.6	67.5	
DPI	11	T6C	2	26.1	53.0	
USA	3	S02	1	37.6	86.5	

## 8 Figures



**Figure 1: Scatter plot of NIR prediction for DPI unit, 6<sup>th</sup> Rib**

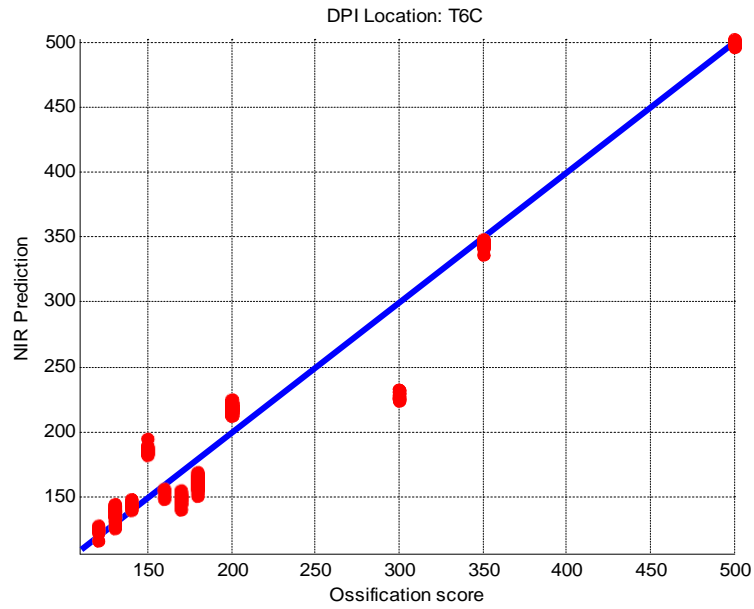


Figure 2: Scatter plot of NIR prediction for DPI unit, T6C location

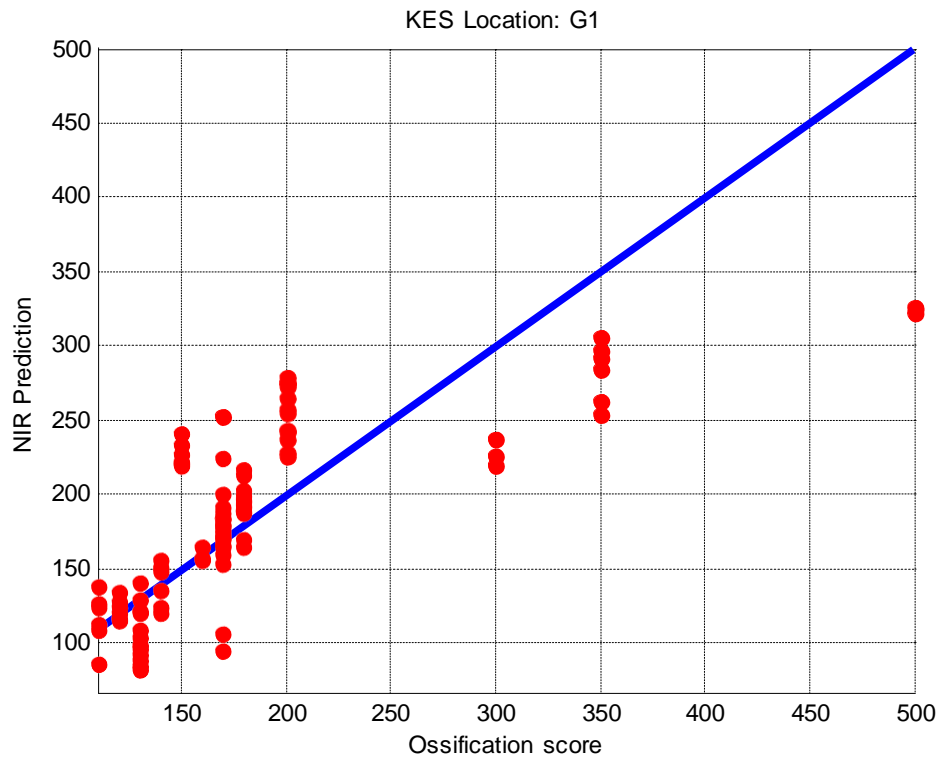


Figure 3: Scatter plot of NIR prediction for KES unit, G1 Location

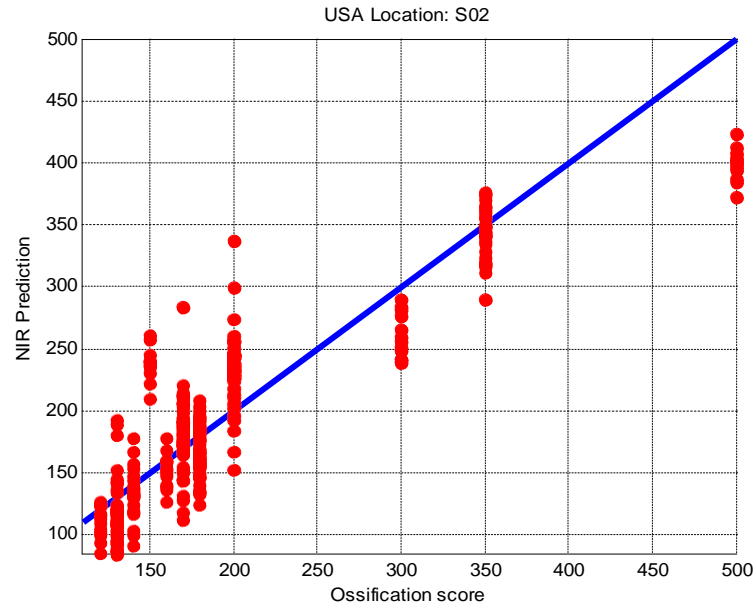


Figure 4: Scatter plot of NIR prediction for USA unit, S02 location

## 9 References

Ossification on sheep by NIR, Matt Kerr and others, DPI.