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Prepared by: Stuart Baud, Matthew Kerr, Oliver Ferdinando, Paul Weston, *Claus Borrgaard
Department of Primary Industries Victoria;
*Danish Meat Research Institute Denmark

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Meat & Livestock Australia Limited
Locked Bag 991
NORTH SYDNEY NSW 2059

On line measurement of muscle glycogen status to predict ultimate meat pH and colour using multi wavelength spectroscopy]

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Executive summary

The project evaluated the potential of multi wavelength spectroscopy as a rapid on line diagnostic tool to cost efficiently measure carcass muscle glycogen and lactate status within 30 minutes post slaughter to predict ultimate pH and meat colour. An early detection method for identifying and grading slaughter stock with high ultimate pH or unacceptable colour on the kill floor would be a valuable first step to optimise the slaughter, chilling, pH and meat colour outcome of slaughter stock of differing glycolytic status.

Multi wavelength spectra and reference samples were collected on a total of 1128 beef carcasses processed at Tasman meat group Brooklyn on account of Coles Myer Pty Ltd. Measurements were collected on two livestock classes cows and young cattle with 0 to 4 teeth. The cattle were processed over 13 separate kill dates between December 2004 and June 2005. A calibration data set of 314 samples were selected from the 1128 beef carcasses measured on the slaughter floor for which laboratory assays for muscle glycogen and lactate levels were conducted. To achieve a wide variation in muscle glycolytic potential at slaughter for each livestock class the samples were selected on the basis of loin muscle ultimate pH measured at 24 hours post slaughter in accordance with pre-prescribed targets. The targeted number of loins with high ultimate pH categories was based on a projected incidence of 10%. The actual incidence of carcasses with, an ultimate pH >5.8 was 9.7%.

Considerable variation existed in the glycolytic status of the cattle processed as evidenced by the reference measurements for muscle glycogen, lactate and calculated glycolytic potential. A wide variation existed between individual carcasses in muscle glycogen (5.90 to 157.96 µmole/gram) lactate (15.91- 74.81 µole/gram) and calculated GP (35.2- 346.7 µmole/gram) values at 30 minutes post slaughter. Similarly, a wide variation also existed between vendor groups of cattle when assessed on the vendor means for muscle glycogen (6.95 to 55.53 µmole/gram) lactate (30.72- 70.33 µmole/gram) and calculated GP (48.99- 156.60 µmole/gram) values at 30 minutes post slaughter.

An ASD multi wavelength spectrometer fitted with a fibre optic probe was successfully used to collect the muscle spectra on the slaughter floor where the chain speed was operating at between 90 —95 carcasses per hour. Prediction model estimates were developed for muscle glycogen, lactate and glycolytic potential from the multi wavelength spectra collected. The prediction models suggest there was only limited useful information in the spectra for predicting muscle glycogen, lactate and calculated glycolytic potential. There was a marked deterioration in the predictive accuracy of the prediction models in this study compared to those generated in an earlier MLA project SNGPI 016.which was conducted under more controlled conditions. Possible reasons for the reduced accuracy of the prediction models are considered to be associated with the placement and size of the optical fibre probe used to collect the spectra. The reference measurement site used (5/6th rib) was selected on the basis that it was the nominated commercial site for quartering the carcass and muscle colour assessment. However at this site there was an increased risk of placing the probe on a fat seam when inserted into the muscle from where the biopsy sample was taken. Taking the muscle biopsy sample and scan from another site was not an option to the commercial collaborator who owned the cattle because of potential carcass downgrading costs associated with taking a biopsy sample. It would also have been desirable to use a larger optical fibre probe to increase the surface area of the muscle from which the spectral data was collected.

Irrespective of the ability or inability of multi wavelength spectroscopy to predict muscle glycogen a more detailed analysis of the reference measurement data found that the original

hypothesis was not supported.- Contrary to a commonly held view within the meat industry, muscle glycogen measured within 30 minutes post slaughter alone did not accurately predict carcass pHu. This was illustrated when a nominated cut off value of below 20 µmole glycogen/ gram muscle or less at 30 minutes post slaughter was used to grade carcasses on the basis of having an expected high pHu of 5.8 or higher. At this cut off value 39.9% of both young cattle and cows in this study would have been misclassified. This would clearly not be an acceptable result. Analysis of the reference data found that muscle glycogen measured at 30 minutes post slaughter was only one of several factors influencing ultimate pH. In addition, the regression model found that calculated glycolytic potential ($P<0.0001$), glycogen depletion rate post slaughter ($P=0.006$) and an interaction of glycogen depletion rate within vendor and animal class ($P=0.006$) were all significant factors influencing pHu. Collectively these variables accounted for 51.34% of the variation in pHu ($P<0.0001$).

In summary the study showed that there was considerable variation in the glycolytic status of stock at slaughter and this had a direct influence on muscle ultimate pH and colour. Whilst the ASD instrument used was capable of operating at commercial chain speeds it was unable to accurately predict muscle glycogen, lactate and calculated glycolytic potential values at the reference site selected. Consequently, the prediction models developed were inferior to those developed in an earlier project conducted under more controlled conditions. However, multi wavelength spectroscopy is considered to still have the potential to provide the meat processing industry with an accurate and rapid on line diagnostic tool to sort carcasses on the kill floor for pHu and colour. It is envisaged to achieve this will require some refinements to the methodology used for collecting the spectral information in a commercial plant. Also. further improvements are required to the prediction model for pHu to improve its accuracy by the identification and incorporation of as yet undefined parameters that influence pHu and colour. The successful development and commissioning of such an on line tool would provide processors and their livestock suppliers with valuable feedback on how to better manage the glycolytic status of their stock pre slaughter to ensure that when processed they comply with the required meat pHu/ colour window.

Contents

1 Background / Description

R&D conducted for the red meat processing industry has defined processing pathways that optimise meat quality attributes. However, inconsistency and variability in meat quality still remain a problem even for the same vendor consignment of stock produced and processed under uniform conditions. Lack of homogeneity in the muscle glycolytic status of stock pre-slaughter is considered a major contributing factor to the observed variability in meat quality in such circumstances. Carcass muscle glycolytic potential at slaughter is known to influence key pre rigor biochemical events that optimise the conversion of muscle to meat. These biochemical changes in turn determine post rigor meat quality attributes including ultimate pH, colour, water holding capacity, retail display life and eating quality. Until recently processors have had few options to provide early identification of susceptible stock to take corrective action. Instead, the first evidence of a meat pH or colour problem is at boning when affected carcasses are downgraded for "dark cutting" or "soft" carcasses. A diagnostic kit to measure muscle glycogen levels has been developed and used in hot boning beef plants in New Zealand. However, being a wet laboratory style method, its perceived lack of user friendliness has restricted industry uptake of the technology as a preventative management tool.

Recent results from an initial feasibility study (MLA project SNGPI 016) support the concept that multi wavelength spectroscopy could be used as an on line tool to measure muscle glycogen levels immediately post slaughter. Furthermore, muscle glycogen levels measured as early as 30 minutes post slaughter appear to be a useful indicator to accurately identify carcasses with a high ultimate pH or unacceptable meat colour. In fact, muscle glycogen level measured within 4 hours of slaughter was a more accurate predictor of pHu than using pre-rigor pH itself. The feasibility study showed that six of the seven (86%) loins with a low muscle glycogen level (<10 µmole/g within 4 hours of slaughter) had a high ultimate pH (pHu >5.85). This compared with only 1 of the remaining 82 loins (<2%) whose muscle glycogen levels exceeded 10 µmole/g.

The project aims to use on-line multi wavelength spectroscopy for the early detection slaughter stock at high risk of having high pHu or unacceptable meat colour. The early detection method would preferably have application pre-slaughter but a more achievable initial step is to develop and validate its application on the kill floor immediately post slaughter. The technology used to underpin both the live animal and kill floor systems is based on the established predictive relationship that exists between muscle glycogen levels and carcass ultimate pH and meat colour. It is proposed both systems will use multi wavelength spectroscopy to measure muscle glycogen levels.

2 Objectives

The Research Organisation will achieve the following objective(s) to MLA's reasonable satisfaction:

- assess the predictive accuracy of multi wavelength spectroscopy to measure pre-rigor muscle glycolytic potential on the slaughter floor and relate this to muscle pHu and colour post slaughter

3 Approach / Methodology

3.1 Animals:

Multi-wavelength spectra and reference samples were collected from 1128 beef carcasses processed at Tasman Meat Group, Brooklyn on account of Coles Myer Pty Ltd. Measurements were collected on two livestock classes; cows and young cattle with 0 to 4 teeth. The cattle were processed over 13 separate kill dates between December 2004 and June 2005. Table 3.1 summarises the distribution of the data collected with regard to ultimate loin pH for each livestock class.

Table 3.1 Summary of the total number of carcasses sampled and their distribution in relation to livestock class, ultimate pH and calibration data set.

Class	Ultimate loin pH	Total no. sampled	Cattle Distribution of samples collected	Calibration data set actual v's target distribution of samples
Cow	<5.80	500	445	96 (100)
	5.80 to 5.99		32	32 (25)
	6.00 plus		23	22 (25)
YP	<5.80	628	574	114 (100)
(0 to 4 teeth)	5.80 to 5.99		27	23 (25)
	6.00 plus		27	27 (25)
	Current total	1128 head		314 (300)

3.2 Calibration data set

A calibration data set of 314 samples was selected from the 1128 beef carcasses measured on the slaughter floor (Table 3.1). To achieve a wide variation in muscle glycolytic potential at slaughter for each livestock class the samples were selected on the basis of loin muscle pHu measured at 24 hours post slaughter in accordance with pre-prescribed targets. The targeted number of loins with high pHu categories was based on a projected incidence of 10%. The actual incidence of carcasses with an ultimate pH >5.8 was 9.7%.

3.3 Carcass Reference Measurements:

Detailed individual carcass information was collected on the 1128 beef carcasses assessed as part of the project. Vendor, dentition, carcass weight, P8 fat depth and sex were recorded for all individual carcasses on the day of kill. Table 3.2 summarises the key reference measurements collected to calibrate with the multi wavelength spectral scan information collected on the loin muscle *(M. longissimus thoracis et lumborum)* at 30 minutes post slaughter. The spectral information was collected whilst the carcass was still on the kill floor at a chain speed rate of 90 to 95 carcasses per hour. A loin muscle biopsy sample was collected at same carcass reference site used to collect the muscle spectra. The biopsy was snap frozen in liquid nitrogen and processed at a later stage to provide reference measurements on muscle glycogen, lactate and calculated glycolytic potential according to Monin and Sellier (1985). A second muscle biopsy

was taken at the same site at 3 hours post slaughter which was used to calculate loin muscle glycolytic rate. Ausmeat meat colour and loin pHu was recorded 20 hours post slaughter. This was measured on the loin muscle quartered at the 516th rib site after a 30 minute bloom.

Table 3.2 Summary of the meat quality traits and time points measured for each loin

Time post slaughter (hours)	MWS scan	Glycogen	Lactate	pH	Aus meat meat colour
0.5					
3		✓	✓		
2 0				✓	✓

Using the statistical techniques described in Section 3.6, the unique spectral information collected on each loin at each nominated time point was calibrated to the reference measurements recorded for the objective meat quality traits detailed below:

Muscle glycogen: Muscle biopsies were collected using an electric drill fitted with a 5mm corer from the **LTL** at 0.5 and 3 hours post slaughter. The biopsies were snap frozen in liquid N at collection and held at —80C until homogenised. A subsample of 0.2g of muscle was homogenised in 1 ml of 30mM HCL using a bead beater homogeniser. Muscle glycogen concentrations were determined from the homogenate, following the enzymatic hydrolysis of glycogen with amyloglucosidase (Dalrymple & Hamm 1973)

Muscle lactate: The muscle biopsy collected for glycogen analysis was also used to measure muscle lactate at the same time points nominated for the glycogen. After thawing and homogenisation lactate was determined in the muscle extract by the method described by Noll 1985.

Muscle glycolytic potential (GP) Glycolytic potential is given by the sum of the main compounds producing lactic acid post mortem and calculated according to the formula proposed by Monin and Sellier (1985).

GP= 2(glycogen) +(glucose) + (glucose 6-phosphate) +(lactate)
Glucose-6-phosphate was not measured in this study as it was assumed the enzymic kit used would have converted it to glucose and hence it would still be included in the GP equation. This decision was base on data from previously reported studies, which suggested that glucose-6-phosphate concentrations were low and consistent contributors to GP (Josell et al 2000). Its exclusion as a separate assay substantially reduced laboratory assay costs

Meat pH: measured at the same time points nominated above and same site used for collecting the MWS spectra using a TPS WP80 pH meter with an 1J44 electrode.

Meat colour: An MSA trained grader assessed loin muscle colour at 516th rib at 20 hours post slaughter after exposure to chiller temperature for 30 minutes.

3.4 Multi-wavelength spectroscopy

Spectral data (350 to 2500nm) was collected using an ASD Field Spec Pro instrument operating in reflectance mode (Figure 1). Spectra: was collected on the 1205 carcasses at 30 minutes then again at 180 minutes post slaughter at the thoracic end of the LTL (5/6th rib site). The optical fibre probe was placed in the same site from where the muscle biopsy was collected. An instrument setting of 20 scans per measurement site was used with spectra collected twice at each time point.
The spectral data collected at two sites (1 & 2) with each analysed independently.

3.5 Statistical Analysis

Spectral data was analysed using the Unscrambler software package. Partial least square regression (PLS) was used to derive calibrations for a number of meat quality measurements from the MWS spectra. An initial analysis of the data compared the prediction models estimates derived using the raw MWS reflectance spectra, first and second derivatives of the spectra the latter generally giving the best results. The second order derivative calculation results in a spectral display of absorption peaks pointing down rather than up. Second derivative can be very helpful in spectral interpretation due to the fact that in this form band intensity and peak location are maintained with those in the log (1/R) spectral pattern, and apparent band resolution enhancement takes place. Consequently, prediction model estimates provided in the report are generated using the second derivative of the spectral data. Also when developing the regression model estimates the jackknife statistical procedure was used. This procedure statistically selects the spectral wavelengths that are most informative to the reference measurement trait of interest and excludes those that are statistically least informative.

The models are validated using a cross validation technique. This method divides the data set into 10 segments. One segment of data was removed from the data set at a time. A regression model is constructed on the remaining segments and then tested on the samples that had been left out. This process was repeated until all segments had been used as a true and independent test for the models created. In this way, all data presented in the following tables allow for a more rigorous test of accuracy rather than listing overall calibration error for all samples in the population.

The predictive accuracy of the models is given by the root mean square error of prediction (RMSEP). This value indicates the average uncertainty that can be expected when predicting Y values for new samples expressed in the same units as the Y variable. The number of PLS factors indicates the complexity of the calibration model with a lower number indicative of a more stable and robust model across data sets.

The SAS system was used to fit all reference measurement data, vendor, kill day, animal class and dentition to a regression model to predict ultimate pH.

Figure 1 Beef carcass loin muscles being measured with the ASD multi wavelength spectrometer fitted with a fibre optic probe on the slaughter floor operating at 90 —95 carcasses per hour.

4 Results

4.1 Muscle glycogen, lactate and glycolytic potential

Reference data Loin muscle glycogen, lactate and calculated glycolytic values measured for the 314 loins selected for the calibration data set at 0.5 and 3 hours post slaughter are summarised in Table 4.1. Considerable variation existed in muscle glycogen lactate and calculated GP values across the 302 beef loins. The overall changes observed post slaughter in the values between the two sampling time points in general reflect the expected trend of a higher initial muscle glycogen levels immediately post slaughter that progressively decline with the onset of rigour. This represents the normal cellular process that occurs post slaughter as muscle glycogen is converted to lactic acid in an anaerobic environment.

Table 4.1 Means, standard deviations, minimum and maximum values for the glycogen, lactate and calculated glycolytic potential of young cattle and cow beef loins at nominated time points post slaughter

Animal class	Meat Quality Trait	No. of Samples	MEAN	SD	RANGE
Young cattle (0-4 teeth)	Glycogen prnole/g				
	0.5hr	158	40.95	26.27	5.90- 157.96
	3 hr	158	19.97	16.53	1.34- 105.38
	Lactate µmole/g				
	0.5 hr	160	43.50	11.23	15.91- 74.81
	3 hr	157	69.57	13.29	34.16- 104.54

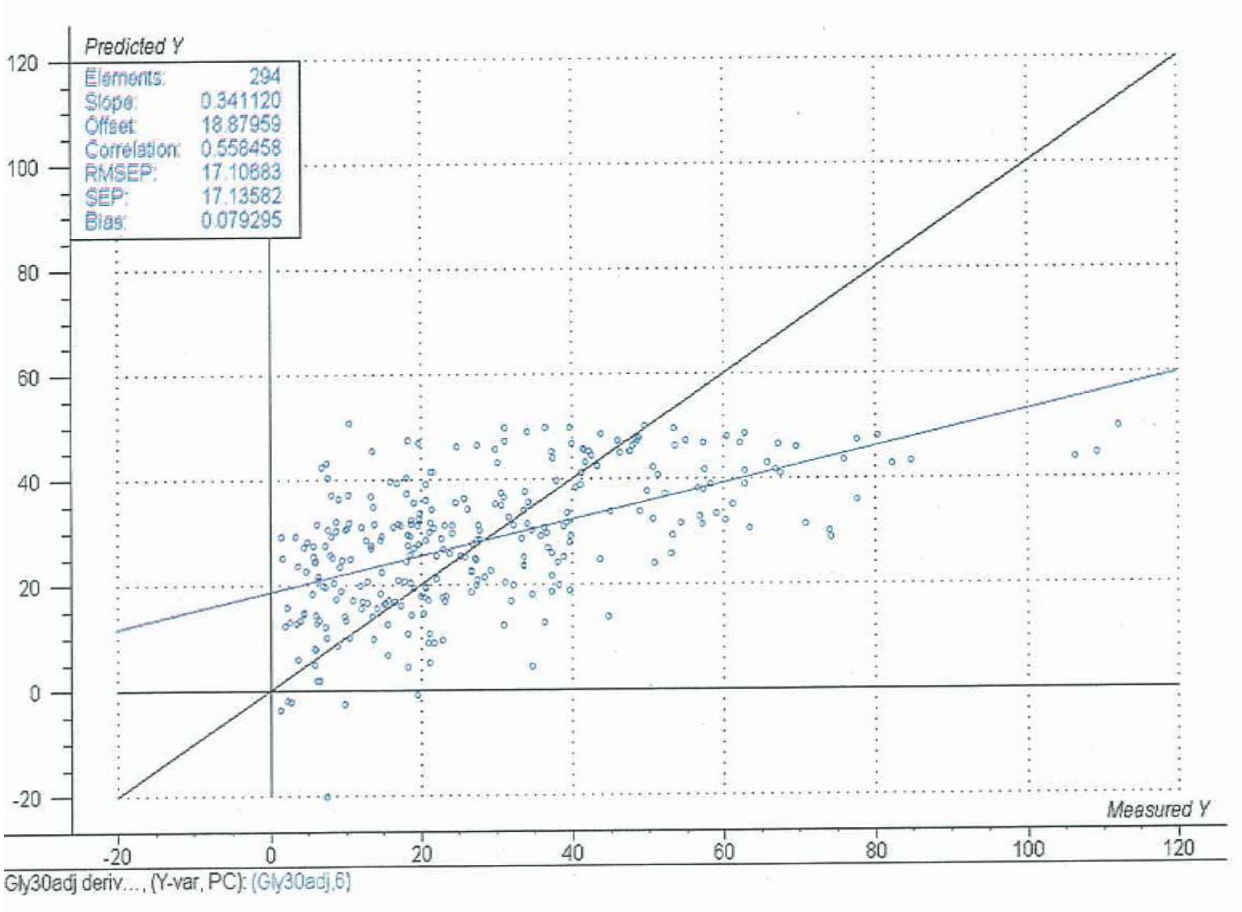
	Glycolytic potential µmole/g				
	0.5 hr	158	125.5	53.3	35.2- 346.7
	3 hr	156	110.0	38.5	40.6- 294.3
Cows	Glycogen µmole/g				
	0.5hr	144	18.60	13.78	1.53-74.41
	3 hr	142	9.66	8.63	0.61- 65.08
	Lactate µmole/g				
	0.5 hr	142	34.95	12.35	5.97-70.66
	3 hr	143	60.13	16.49	20.36- 106.71
	Glycolytic potential µmole/g				
	0.5 hr	142	72.55	30.73	18.86- 173.63
	3 hr	141	79.49	25.72	26.30- 154.88

Considerable variation also existed in muscle glycogen lactate and calculated GP values across the 302 beef loins when analysed on vendor groups (Table 4.2). The ratio of glycogen at 30 minutes (gly0.5) to calculated glycolytic potential (GP 0.5) provides an indication of the level of glycogen available for glycolysis at 30 minutes post slaughter as a proportion of total initial glycolytic reserves (GP 0.5) reserves available at slaughter when the metabolic changes occurring in muscle cell essentially became a closed system.

Table 4.2 Vendor means for pHu ,glycogen (gly) at 0.5 and 3hours , glycogen decline from 0.5 to 3 hours, calculated glycolytic potential (GP) at 0.5 hours and the ratio of glycogen to calculated GP at 0.5 hours post slaughter

Vendor	**No. of head**	**pHu**	**Gly 0.5**	**Gly 3**	**Gly decline 0.5 to 3hrs**	**%Gly decline 0.5 to 3hrs**	**GP 0.5**	***Ratio*** **Gly0.5 to GP0.5**
Time post slaughter (hours)			0.5	3			0.5	0.5
Young cattle								
Abco	35	5.77	28.56	18.42	-10.1	-35.4	104.03	27.5
Charlton	38	5.65	55.53	19.57	-35.96	-64.7	156.60	35.5
Merryville	37	5.81	44.21	20.26	-23.95	-54.2	133.40	33.1
Yambinya	24	5.93	30.09	21.39	-8.70	-28.9	92.30	32.6
Cows								
Aldrick	17	6.17	10.23	5.70	-4.53	-44.3	51.17	20.0
Alexandra	5	5.92	17.68	19.73	+2.05	+11.6	62.35	28.3
Corryong	7	6.08	6.95	6.58	-0.37	-5.3	48.99	14.2
Eulameet	33	5.74	21.49	10.85	-10.64	-49.5	80.76	26.6
Harvey	34	5.69	17.4	6.30	-11.10	-63.7	58.10	29.9
Waine	13	5.74	20.16	12.16	-8.00	-39.7	84.37	23.9

Figure 2 Prediction of muscle glycogen at 30 minutes post slaughter based on the MWS spectra



4.2 MWS prediction models

The prediction model estimates for muscle glycogen, lactate and glycolytic potential from the multi wavelength spectra collected are summarised in Tables 4.3, 4.4 & 4.5. The prediction models suggest there was only limited useful information in the spectra for predicting muscle glycogen, lactate and calculated potential. There was a marked deterioration in the predictive accuracy of the current models compared to those generated in project SNGPI 016 which was conducted under more controlled conditions. The predictive accuracy of the model is optimised when the regression co-efficient approaches 1, the standard error of prediction (SEP) is low, the ratio of the SEP to the standard deviation of the predicted trait approaches 0.3 and the number of factors contributing to the PLS model is low.

Glycogen: The prediction models developed for muscle glycogen using the spectral data collected at 30 minutes post slaughter are summarised in Table 4.3. All the prediction models had low r values and high associated errors of prediction Taking the first derivative of the spectral data and/or removing samples if the calculated glycolytic potential values at 0.5 and 3hours post slaughter differed by more than 50% did result in some minor improvement in the prediction model values for correlation co-efficient (r), standard error of prediction (SEP) and the ratio of SEP to the standard deviation (SD).

Table 4.3 Prediction model estimates for muscle glycogen (30 minutes post slaughter)

Data treatment	Scan (no. scanned)	Model estimates			
		R	SEP	SEP/SD (SD=23.98)	PLS factors
Original	1 (302)	0.27	23.14	0.96	3
	2 (302)	0.35	22.45	0.94	3
Derivative	1 (302)	0.36	22.36	0.93	2
	2 (302)	0.39	22.11	0.92	3
Derivative less 8 outliers	1 (294)	0.56	17.11	0.71	6
	2 (289)	0.56	15.76	0.66	3
Derivative plus GP 0.50:GP3 >50% removed	1 (261)	0.51	18.65	0.78	8
	2 (261)	0.33	20.46	0.85	1

Lactate: The prediction models generated for muscle lactate also lacked accuracy as reported for muscle glycogen (Table 4.4) as indicated by the ratio of SEP to SD which as with muscle glycogen was approaching 1. It is generally considered that if the standard error of prediction of the instrument is equal to the standard deviation of the trait being measured then the value of the prediction is very low.

Table 4.4 Prediction model estimates for muscle lactate

Data treatment	Scan (no. scanned)	Model estimates			
		r	SEP	SEP/SD (SD=12.50)	PLS factors
Original	1 (302)	0.49	10.97	0.88	10
	2 (302)	0.35	11.73	0.94	3
Derivative	1 (302)	0.52	10.76	0.86	9
	2 (302)	0.52	10.67	0.85	4
Derivative less outliers	1 (294)	0.55	9.94	0.80	4
	2 (293)	0.52	10.24	0.82	6
Derivative plus GP 0.50:GP3 >50% removed	1 (261)	0.58	9.64	0.77	5
	2 (261)	0.42	10.69	0.86	3

Glycolytic potential Prediction models developed for glycolytic potential (GP) were also of low accuracy (Table 4.5) as reported for muscle glycogen and lactate. This is attributed to the fact that GP was calculated using both the glycogen and lactate muscle reference values.

Table 4.5 Prediction model estimates for calculated glycolytic potential (GP)

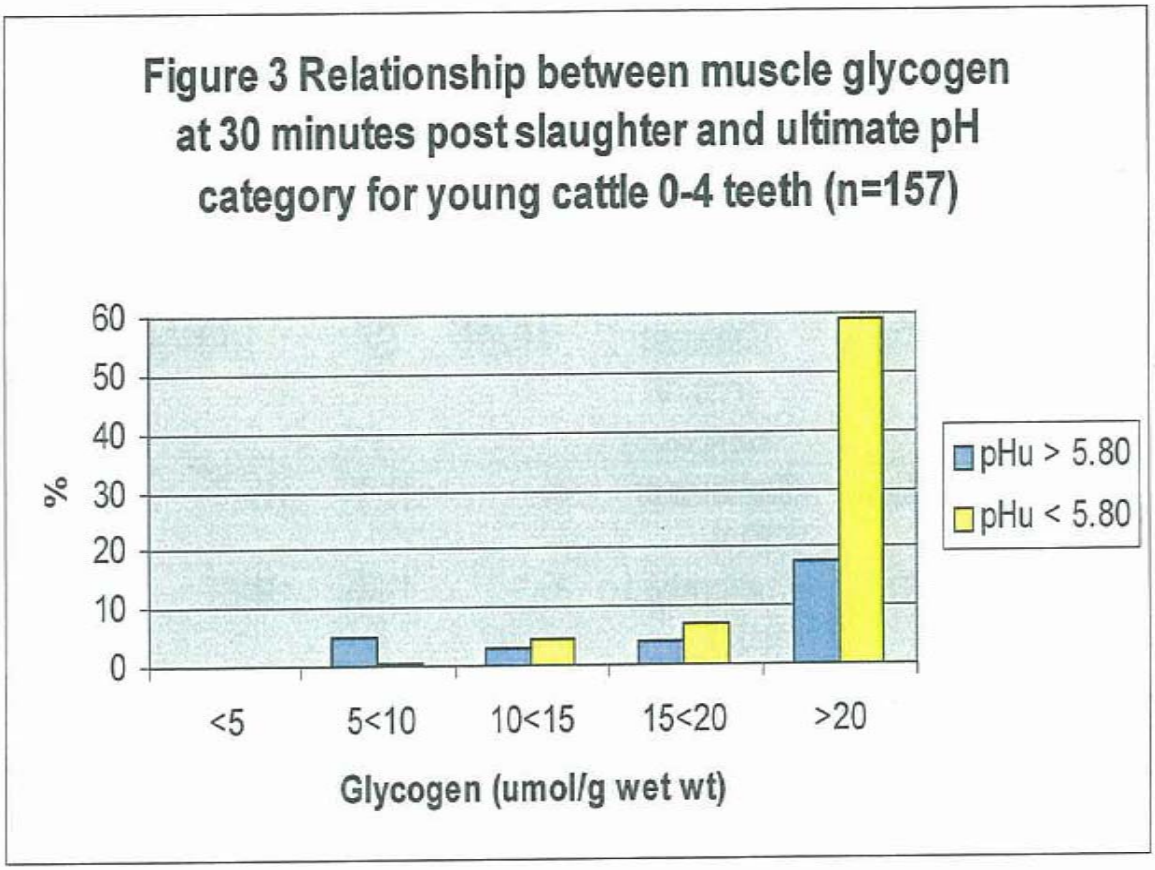
Data treatment	Scan (no. scanned)		Model estimates r	SEP	SEP/SD (SD=51.4)	PLS factors
Original	1		0.34	48.36	0.94	1
	2		0.38	47.56	0.93	3
Derivative	1		0.35	48.23	0.94	2
	2		0.44	46.13	0.90	2
Derivative less outliers	1	(293)	0.64	35.53	0.69	6
	2	(285)	0.66	31.64	0.62	6
Derivative plus GP 0.50:GP3> 50% removed	1	(257)	0.59	34.98	0.68	7
	2	(261)	0.44	41.63	0.81	2

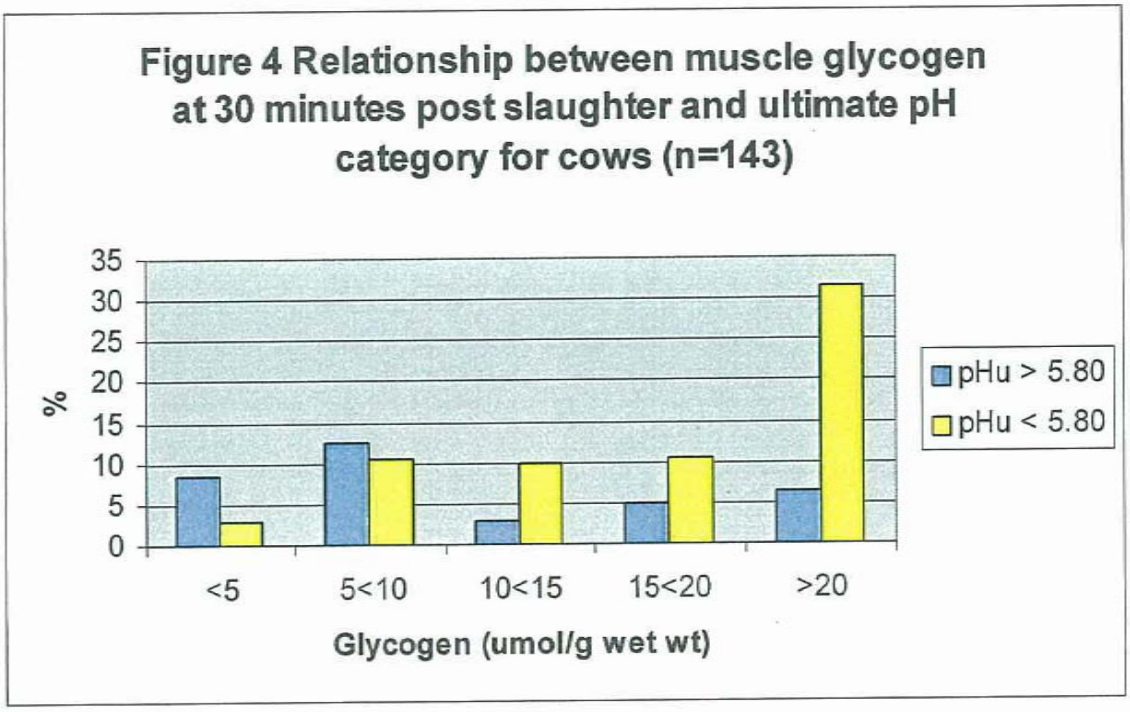
4.3 Loin muscle pH & colour

Loin muscle pH and colour was measured at the 516th rib site at around 20 to 24 hours post slaughter (pHu). Table 4.6 summarises the means, standard deviations and range for muscle pH and colour for the total data set (1128 head and calibration data set (302 head)

Table 4.6 Means, standard deviations, minimum and maximum values for the meat colour and pH of the young cattle and cow loins at nominated time points post slaughter

Meat Quality Trait	Class	Data set (no. of samples)	MEAN	SD	RANGE
Ausmeat meat colour (24hrs)	Cows	Calibration (151)	4	1.12	1C- 7
	YC	Calibration (151)	3	1.22	1B-6
Meat pH (24 hrs)	Cows	Total (500)	5.62	0.20	
		Calibration (151)	5.78	0.29	5.40- 7.15
	YC	Total(628)	5.59	0.19	
		Calibration (163)	5.76	0.29	5.35- 6.98





Irrespective of the ability of multi wavelength spectroscopy to predict muscle glycogen the actual glycogen reference measurement taken at 30 minutes post slaughter alone did not

support the original hypothesis that muscle glycogen measured within 30 minutes post slaughter could be used to accurate predict carcasses on their ultimate pH. This is illustrated if 20 µmole glycogen per.gram of wet muscle weight or less at 30 minutes post slaughter is nominated as a cut off value to sort young cattle into a high ultimate pH category (pHu> 5.8). If this cut off value is used 39.9% of young cattle carcasses would have been mis-classified. Of the 39.9% carcasses misclassified 17.8% had muscle glycogen levels over 20 µmole/g but still had an ultimate pH over 5.8 whilst 12.1% had muscle glycogen levels under 20 µmole/g but still had an ultimate pH under 5.8 (Figure 3).

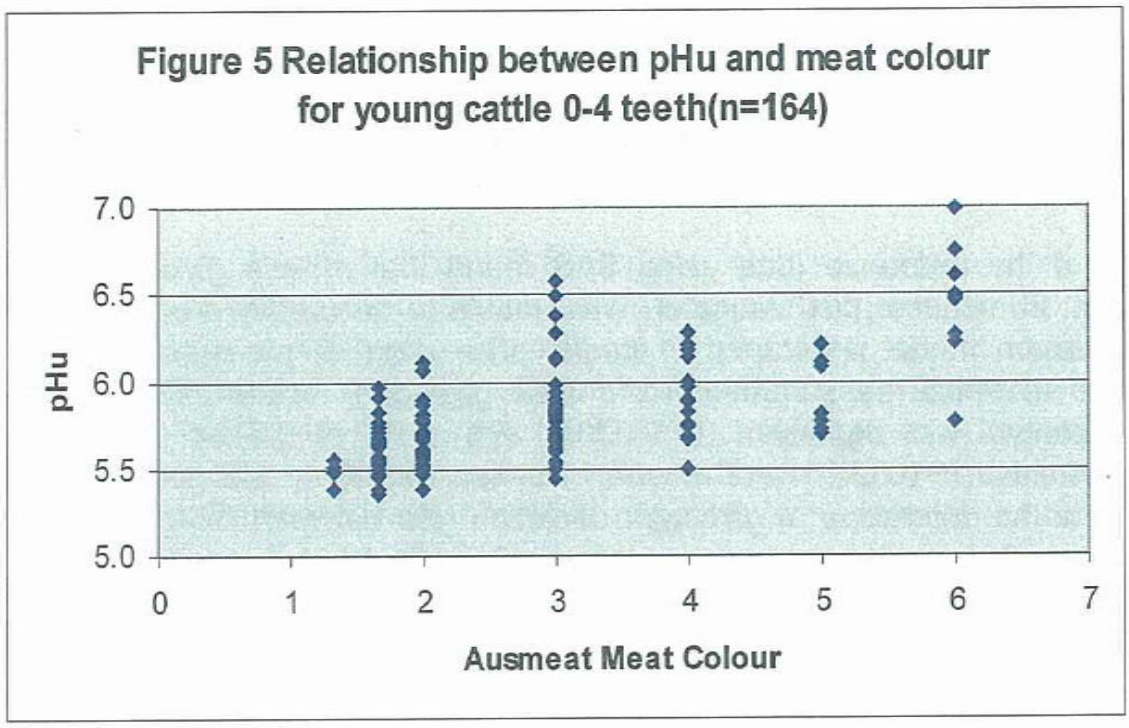
Using the same cut off value for cows coincidently also resulted in 39.9% being misclassified. However, 6.3% were misclassified because whilst their glycogen levels were over 20 µmole/g they had an ultimate pH over 5.8. Conversely, the balance (33.6%) had muscle glycogen levels under 20 .µmole/g but had an ultimate pH under 5.8 (Figure 4)

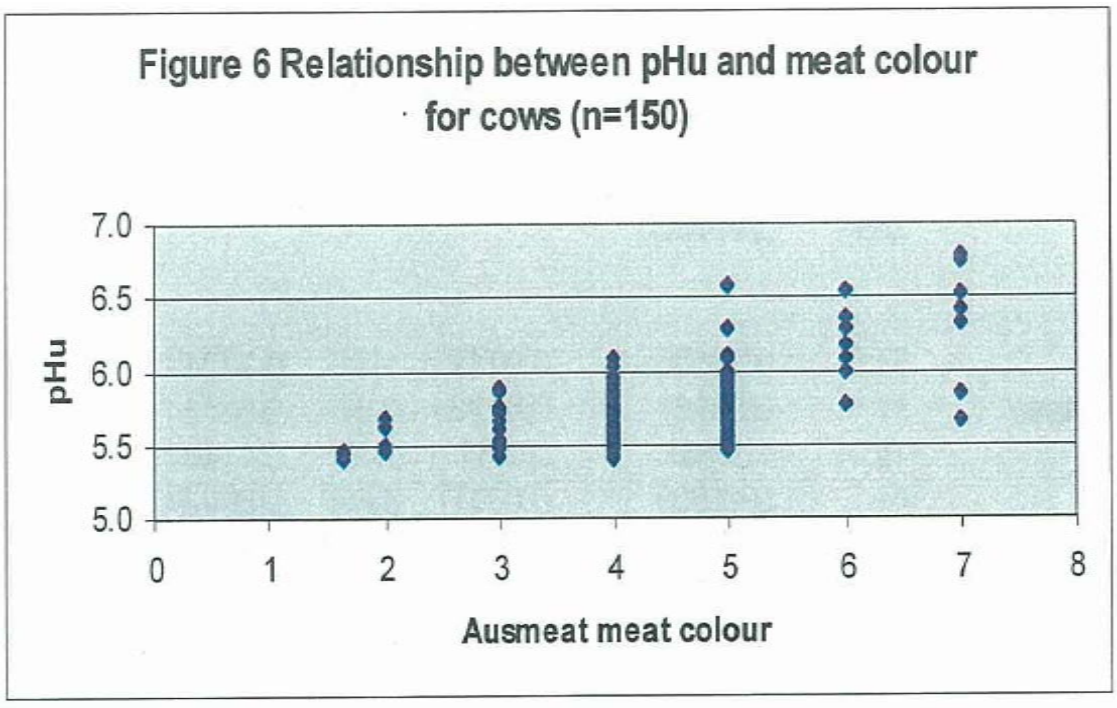
Re-analysis of the reference data using SAS found that muscle glycogen alone (measured at 30 minutes post slaughter) was unable to accurately predict ultimate pH. A regression model developed to predict pHu using all measured variables including the reference measurements of muscle glycogen, lactate and calculated glycolytic potential was significant (P<0.0001) with a R2 of 0.5134 (Table 4.7). Glycolytic potential (P<0.0001) and muscle glycogen depletion rate post slaughter (P<0.006) and the interaction of glycogen depletion rate between 0.5 and 3 hours post slaughter within vendor and animal class (P=0.0058) were all significant factors influencing pHu. The effect of muscle glycogen at 0.5 hours post slaughter approached significance (P=0.055) the effects of vendor, dentition, livestock class and kill day did not significantly effect pHu.

Table 4.7 Summary of the factors and their statistical significance fitted to the regression model to predict ultimate pH (R2 =51.34%)

Source		DF	Sum of squares	Mean Square	F value	Pr
Model		64	12.7812	0.1997	3.63	**<0.0001**
Error		220	12.1140	0.0551		
Total		284	24.8953			
Kill day		3	0.2156	0.0719	1.31	0.2736
Livestock class		1	0.0123	0.0123	0.22	0.6372
Vendor		15	1.2168	0.0811	1.47	0.1168
Dentition		4	0.1509	0.0377	0.69	0.6028
GP 0.5hrs		1	0.8994	0.8994	16.33	**<0.0001**
Glycogen 0.5hrs		1	0.2046	0.2046	3.72	**0.0552**
Glydifference0.5-3hrs		1	0.1379	0.1379	2.51	0.1149
Glydifference to3hrs						
*Vend(Class)	0.5	25	2.6919	0.1077	1.96	**0.0058**

There was a positive relationship between ultimate pH and meat colour for both young cattle (Figure 5) and cows (Figure 6) but pHu alone could not accurately sort carcasses on colour. There was a negative relationship between glycolytic potential at 30 minutes post slaughter and meat colour (Figures 7 & 8) but it too could not accurately sort carcasses on colour.





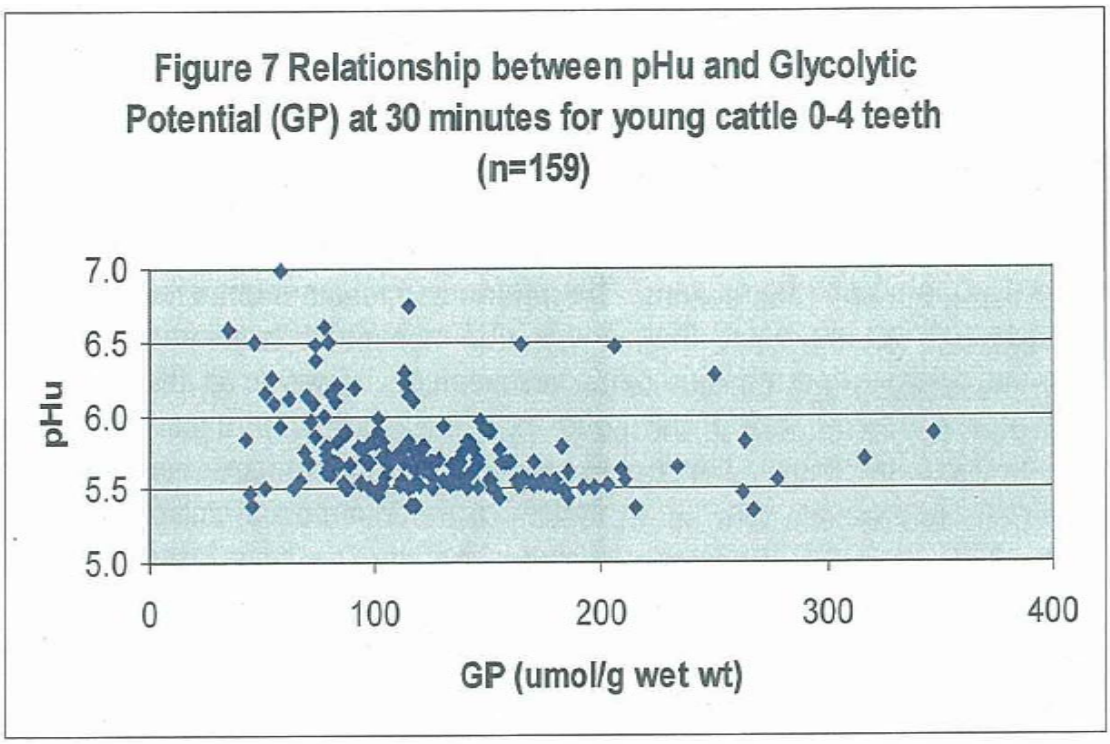


Figure 7 Relationship between pHu and Glycolytic Potential (GP) at 30 minutes for young cattle 0-4 teeth (n=159)
pHu
7.0
6.5
6.0
5.5
5.0
0
100
200
300
400
GP (umol/g wet wt)

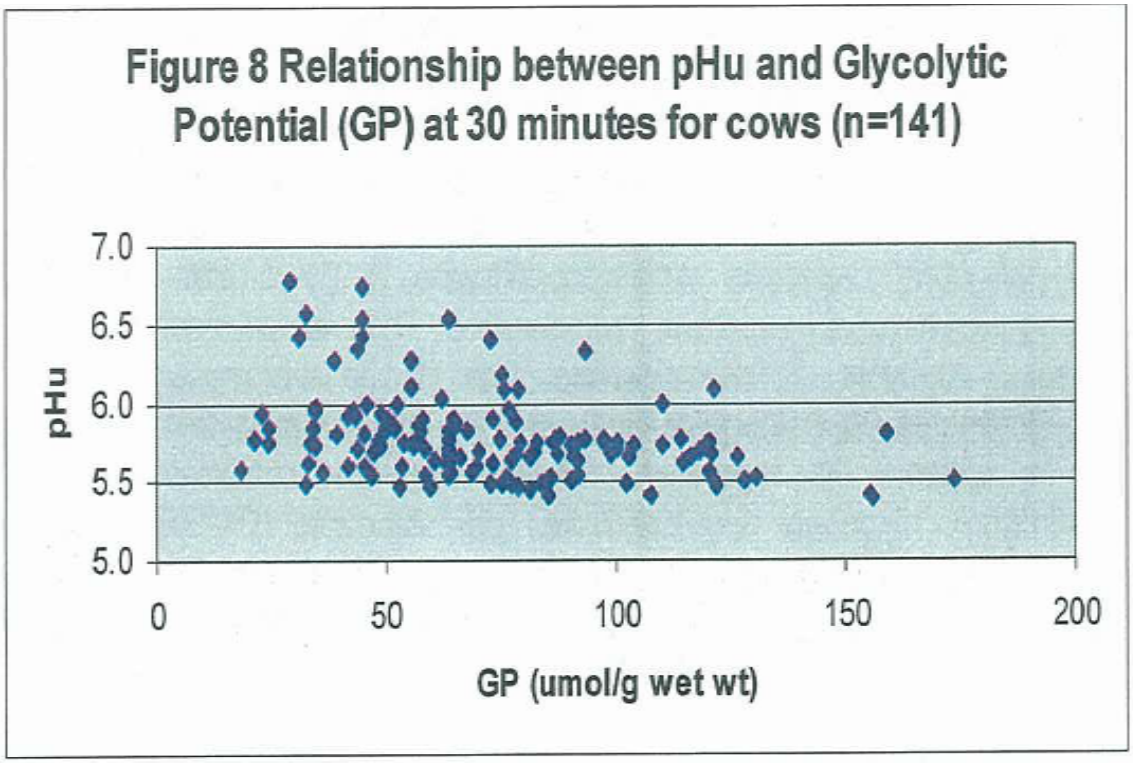


Figure 8 Relationship between pHu and Glycolytic Potential (GP) at 30 minutes for cows (n=141)
pHu
7.0
6.5
6.0
5.5
5.0
0
50
100
150
200
GP (umol/g wet wt)

5 Discussion

Considerable variation existed in the glycolytic status of the cattle processed as evidenced by the reference measurements for muscle glycogen, lactate and calculated glycolytic potential. A wide variation existed between individual carcasses in muscle glycogen (5.90 to 157.96 µmole/gram) lactate (15.91- 74.81 µmole/gram) and calculated GP (35.2- 346.7 µmole/gram) values at 30 minutes post slaughter. Similarly, a wide variation also existed between vendor groups of cattle when assessed on the vendor means for muscle glycogen (6.95 to 55.53 µmole/gram) lactate (30.72- 70.33 µmole/gram) and calculated GP (48.99- 156.60 µmole/gram) values at 30 minutes post slaughter.

The ASD multi wavelength spectrometer fitted with a fibre optic probe was successfully used to collect the muscle spectra on the slaughter floor where the chain speed was operating at between 90 —95 carcasses per hour. However, there was a marked deterioration in the prediction model estimates developed for muscle glycogen, lactate and glycolytic potential from the multi wavelength spectra collected in this study compared to those derived from an earlier MLA study (SNGPI016), which was conducted under more controlled conditions. In this study the prediction models estimates were well short of the estimates required to ensure the instrument has a high predictive accuracy. This occurs if the regression co-efficient approaches 1, the standard error of prediction (SEP) is low, the ratio of the SEP to the standard deviation of the predicted trait approaches 0.3 and the number of factors contributing to the PLS model is low. Possible reasons for the reduced accuracy of the prediction models are considered to be associated with the placement and size of the optical fibre probe used to collect the spectra. The reference measurement site used (5/6th rib) was selected on the basis that it was the nominated commercial site for quartering the carcass and muscle colour assessment. However at this site there was an increased risk of placing the probe on a fat seam when inserted into the muscle from where the biopsy sample was taken. Taking the muscle biopsy sample and scan from another site was not an option to the commercial collaborator who owned the cattle because of potential carcass downgrading costs associated with taking a biopsy sample. It would also have been desirable to use a larger optical fibre probe to increase the surface area of the muscle from which the spectral data was collected.

Irrespective of the ability or inability of multi wavelength spectroscopy to predict muscle glycogen a more detailed analysis of the reference measurement data found that the original hypothesis was not supported. Contrary to a commonly held view within the meat industry, muscle glycogen measured within 30 minutes post slaughter alone did not accurately predict carcass pHu. This was illustrated when a nominated cut off value of below 20 µmole glycogen/ gram muscle or less at 30 minutes post slaughter was used to grade carcasses on the basis of having an expected high pHu of 5.8 or higher. At this cut off value 39.9% of both young cattle and cows in this study would have been misclassified. This would clearly not be an acceptable result. Analysis of the reference data found that muscle glycogen measured at 30 minutes post slaughter was only one of several factors influencing ultimate pH. In addition, the regression model found that calculated glycolytic potential ($P<0.0001$), glycogen depletion rate post slaughter ($P=0.006$) and an interaction of glycogen depletion rate within vendor and animal class ($P=0.006$) were all significant factors influencing pHu. Collectively these variables accounted for 51.34% of the variation in pHu ($P<0.0001$).

In summary the results indicate that before multi wavelength spectroscopy can be used by the meat processing industry to accurately sort carcasses on the kill floor for pHu and colour some further refinements are required. It is recommended that the methodology used for collecting the spectral information in a commercial plant be reviewed to more precisely define the standard

operating procedures with regard to carcass muscle reference site(s) used, optical fibre probe size and placement position to optimise the quality of the spectra collected. Also,. further improvements are required to the prediction model for pHu to improve its accuracy by the identification and incorporation of as yet undefined parameters that influence pHu and colour in addition glycogen, lactate and calculated glycolytic potential. The successful development and commissioning of such an on line tool would provide processors and their livestock suppliers with a valuable on line diagnostic tool to better manage the glycolytic status of their stock pre slaughter to ensure that when processed they comply with the required meat pHu/ colour window.