final report

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Use of Raman Spectroscopy for the Measurement of Meat Quality

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Milestone

Conduct Experiment 1: - Data collection phase 2 and analysis. NSW DPI to provide a final report summarising findings from experiment 1.

Abstract

The data collection phase of the first experiment is almost complete. Testing of shear force, cooking loss, sarcomere length and pH has been completed on all samples collected in the first experiment. Analysis of particle size is yet to be completed in the Cowra lab due to an instrument malfunction, with only a few samples remaining to be processed. Some results from the histology samples that were sent away for fixing and sectioning at the School of Anatomy, Physiology & Human Biology, University of Western Australia have come in and images are being processed. It was found that shear force of the *semimembranousus* (SM: topside) after 5 days of ageing could be predicted from Raman spectra taken at 1 day of ageing (R^2 = 0.27: RMSEP = 11.48), but that Raman spectra taken on the *Longissimus thoracis lumborum* (LL: loin) were not informative. Of particular interest was the finding that using Raman spectra to predict shear force in the topside after 5 days of ageing was more precise (lower RMSEP) than using traditional indicators of shear force such as pH, sarcomere length or cooking loss. Two papers have also been submitted with the approval of AMPC to the 59th *International Congress of Meat Science and Technology* to be held in Turkey.

Project objectives

1. Establish whether measurement of sheep meat at 1 day post-mortem using a Raman hand held probe is useful for predicting shear force after an ageing period of 5 days.
2. Establish whether the probe can be used to predict shear force of 5 day aged meat.
3. Establish what biochemical and biophysical changes the probe is detecting in relation to tenderisation and to explore the potential of the probe to provide measures of other traits such as fatty acids and intramuscular fat.

Success in achieving milestone

The data collation phase for the first experiment is almost complete, but the primary traits of interest have been tested. Data analysis for shear force, cooking loss and sarcomere length in relation to Raman spectra has been completed. Two papers have been submitted with the approval of AMPC to the 59th *International Conference of Meat Science and Technology* to be held at Turkey in August (Appendix 1 and 2). Further to this, three new experiments have been recently conducted over the last two months while the Raman spectroscopic probe was in Australia. The project is making good progress, but there is significant work still to be undertaken to establish the merits of Raman spectroscopy for application to the measurement of meat quality traits.

Overall progress of the project

The project is progressing very well and much progress has been made. It has been shown that the measurement of the SM in the topside using Raman spectroscopy may offer potential for prediction of shear force in 5 day aged meat. There have been extensive discussions on how to analyse spectra and how to ensure that robust models are derived and review of the literature indicates that many studies are published with models that are over fitted and/or which have not be independently validated.

Recommendations

To supplement the work undertaken in Experiment 1, 2 new experiments have been planned (the experimental protocol is outlined in appendix 3). These experiments will allow the following;

1. Validate of the models developed in Experiment 1 for predicting shear force in 5 day aged topsides.
2. Examination of the importance of repetitions at each measurement site.
3. Examination of the potential for pre-rigor scanning of the topside *in situ*. This is designed to also include measures of pH decline based on promising results from work published by the German collaborators in pork Scheier, R., Petzet, A., Bauer, A. and Schmidt, H. (2013). Hand-held Raman system for an early postmortem detection of pH and drip loss of pork meat. *Proc. 58th International Congress of Meat Science and Technology.* (*in press*), Izmir, Turkey and Scheier, R. and Schmidt, H. (2013). Measurement of the pH value in pork meat early post-mortem by Raman spectroscopy. Applied Physics B, (*in press*).
4. Examination of the importance of location and orientation in the measurement of the topside (SM) on the prediction of meat traits.

Additionally, a journal paper outlining the results from the first Experiment will be completed.

Appendices
Appendix 1.

Predicting tenderness of fresh ovine *semimembranous* using Raman Spectroscopy

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Abstract – A hand held 671nm wavelength Raman probe was used to predict shear force of raw intact lamb muscle at day 5 post mortem. Samples (n = 80) of *m. semimembranosus* (topside) from different carcases were measured 24h post mortem and after a further 4 days ageing at 1˚C. At 5 days post mortem shear force (SF) and traditional indicators of shear force (cooking loss, sarcomere length and pHu) were measured. SF values were regressed against Raman spectra using partial least squares (PLS) and against traditional indicators using ordinary least squares regression. For SF prediction using spectra taken 24h post mortem, the root mean square error of prediction (RMSEP) was 11.48N and the correlation between observed and predicted values (R^2_{cv}) was equal to 0.27. Corresponding values for spectra measured 5 days post mortem were RMSEP of 12.20N and R^2_{cv} = 0.17. This is the first study to measure fresh lamb topside using Raman Spectroscopy and there is evidence to suggest Raman spectra taken 24h post mortem is a better predictor of shear force than sarcomere length, cooking loss and/or pHu at 5 days post mortem. Further work to validate the models generated in this study is required to establish the potential benefits of Raman spectroscopy.

Key Words – shear force, meat quality assessment, sheep

I. INTRODUCTION

Tenderness is deemed the most important factor in determining consumer acceptance. It is determined by the interactions between myofibrils and connective tissue and the extent of degradation of myofibrils [1]. Due to this importance, considerable research has focused on the ability of technologies to objectively measure tenderness. A review of such technologies has highlighted Raman Spectroscopy as having the potential to be used for online assessment of meat quality traits [1] as it is non-invasive, rapid, non-destructive and is not sensitive to varying water content [2]. Recent research has not overlooked these advantages and several studies have been conducted to determine the ability of Raman Spectroscopy to predict sensory traits of beef silverside [3], assess the effect of ageing on pork [4] and investigate the relationship between cooking loss and shear force in lamb [5]. While informative, these studies are limited in terms of online application by the cooking, freezing or homogenisation of samples and the use of bench top devices. Further to this no studies have been reported on the

measurement of unfrozen meat by Raman Spectroscopy and then measurement of shear force and other traits on the same piece of meat.

This study reports, for the first time, the potential of a Raman hand held device to predict the tenderness of fresh intact lamb *m. semimembranosus* (topside).

II. MATERIALS AND METHODS

One *m. semimembranosus* (SM) was collected from each of 80 lamb carcasses over 4 days (20 per day). Raman spectra were measured with a 671nm Raman hand held sensor head [6] with 70mw laser power and a 3.75s integration time with no repetitions. At 1 day post mortem each SM had the silverskin removed before being scanned 10 times, perpendicular to the muscle fibres. After measurement the SMs were vacuum packed and held at 1˚C for 4 days. At 5 days post mortem the SM were removed from the vacuum pack and allowed to 'bloom' for 2 hours before a freshly cut surface was rescanned. At 5 days post mortem, sections were excised (mean 65g) for shear tests with a Lloyd texture analyser, as previously described [7]. Samples were weighed before and after cooking to determine cooking loss. Ultimate pH (pHu) was measured using a homogenate method [8] and sarcomere length determined by the laser diffraction method [9] on samples at 1 day post-mortem.

The 10 Raman spectra per SM were averaged then standardised by dividing each by its l_2-norm (square root of sum of squared intensities). The models for predicting shear force which included Raman spectra were fitted using partial least squares (PLS) regression analysis, using R computer software [10]. The number of latent variables (LV) was determined using 20 replications of 8-k fold cross validation and selecting the model with the minimum average root mean square error of prediction (RMSEP). Models not using Raman were fitted using ordinary regression least squares regression. Predictions for each observed shear force value, for each SM sample, were obtained using the Leave-One-Out (LOO) cross validation method.

III. RESULTS AND DISCUSSION

In Table 1, summary results for shear force, sarcomere length, pHu and cooking loss measurements are given. At 5 days post mortem, shear force measurements had a large range (Table 1) but none were below 27N, which indicates very tender samples [11].

Table 1. Mean, standard deviation (SD), and range for shear force (SF; N), cooking loss (CL; %), sarcomere length (SL; µm) and pHu.

Trait	Mean	SD	Range (min, max)
Shear Force (N)	51.4	13.1	29.2 - 78.4
Cooking Loss (%)	19.2	3.7	0.24- 28.8
Sarcomere Length (µm)	1.70	0.11	1.46 - 1.99
pHu	5.61	0.11	5.52 - 6.23

The root mean square error of prediction (RMSEP) values for prediction of shear force using the traditional indicators traits and Raman spectra are summarized in Table 2.

Table 2. RMSEP for models using traditional indicators and/or Raman spectra to predict shear force (N) of 5 day aged lamb topside.

Model Covariates	RMSEP
Cooking Loss (CL)	13.34

Sarcomere Length (SL)	13.23
pHu	13.75
CL, SL and pHu	13.90
Raman Spectra (1 day)	11.48
Raman Spectra (5 day)	12.20
Raman Spectra (1day) + CL, SL and pHu	12.02
Raman Spectra (5 day) + CL, SL and pHu	13.00

Based on the RMSEP criterion, the best model of those considered for prediction of shear force (Table 2) uses the Raman spectra measured at 1 day post mortem (RMSEP = 11.48, LV= 3). The squared correlation (R^2_{cv}) between cross validated predicted and observed shear force values is R^2_{cv} = 0.27 (Fig 1).

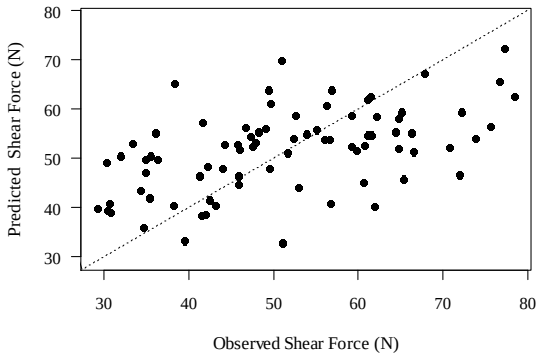

Figure 1. Cross validated prediction of shear force values (N) at 5 days post mortem with Raman spectra collected on day 1 post mortem and analysed with 3 latent vectors.

Although changes to Raman spectra due to ageing are complex and are outside the scope of this paper, results reported here indicate the prediction of shear force using Raman spectra taken on 1 day post mortem is less variable (R^2_{cv} = 0.27; RMSEP= 11.48) than the prediction using Raman spectra collected 5 days post mortem (R^2_{cv} = 0.17; RMSEP = 12.20). As muscle is a closed system, the attributes of muscle which determine the effects of proteolysis and the subsequent amount of myofibrillar degradation during ageing are predetermined by the biophysical and biochemical properties at processing. Whether the ageing process is a result of the interaction of actin and myosin, a change in ionic strength or the action of calcium ions on proteins [12], it is hypothesized that this change or the loss of myofibrillar structure weakens the Raman signal when spectra are taken at 5 days. If changes in ionic strength during ageing are affecting the ability of Raman to predict shear force, collecting spectra as the muscle enters *rigor* may improve predictions because this is the period in which initial changes to electrochemistry occur [12].

None of the traditional indicators were significant predictors of shear force alone or jointly (P > 0.05) and combining all three measured with Raman spectra gave no improvement to the predictability of the model. Despite some previous studies linking these indicators to shear force [11, 13], results reported agree with others that suggest these indicators do not explain large amounts of variation in shear force [14]. A wider range of shear force values with some below < 27N would improve this outcome. It however also important to acknowledge that aging weakens the relationship established at *rigor* [14] so it is not so surprising that sarcomere length wasn't a significant predictor of shear force in aged meat (Fig 2).

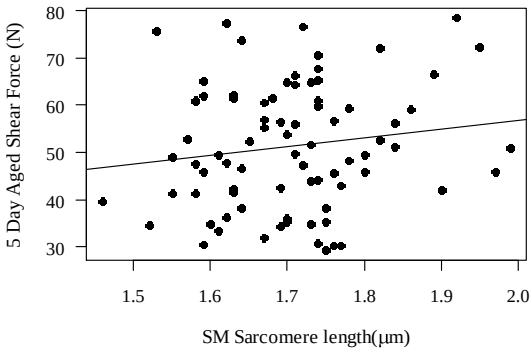

Figure 2.The relationship between shear force values (N) and sarcomere length (μm) for SM samples after 4 days of ageing.

The predictions reported in this paper have lower accuracy compared to coefficients of determination previously reported [3-5]. However, other studies have measured meat that was frozen and thawed, causing myofibrillar disintegration and increased water movement within the muscle [15]. It is hypothesised that increases in shear force prediction accuracy may occur when measuring frozen and thawed meat as Raman is sensitive to changes induced by freezing and thawing [16].

It should also be acknowledged that like other spectroscopic technologies, Raman Spectroscopy is sensitive to variations in experimental design including sampling and sample handling, statistical analysis and equipment parameters [17]. Therefore, it is difficult to compare the results reported against other studies as there is little accordance between experimental design and statistics reported. An example is the difference in integration times used, as previous studies reported times between 2 seconds and 6 minutes [3-5, 18]. Since Raman scattering is relatively weak, spectra of biological samples may be compromised by noise or non-Raman signals and background radiation [17]. Consequently, longer integration times and repetitions or more scans may improve the prediction of shear force by improving the signal to noise ratio. While a 6 minute integration time or 25 scans per sample will not suit an online application, increasing the total accumulation time by increasing integration time or including repetitions may improve the prediction of shear force. Overall, the sensitivity of Raman to experimental parameters emphasises the need for validation of prediction models generated on completely independent samples and as yet no such study has been conducted.

IV. CONCLUSION

Overall it is difficult to determine the ability of Raman spectroscopy to predict shear force values of intact lamb samples, as there is currently no opportunity to evaluate these results reported against other studies which have the same experimental design, equipment and statistical analysis. Therefore, the accuracy and robustness of these predictions need to be validated and the impact of variations in scanning, sampling and chemometric analysis needs to be determined. However, this study suggests that use of Raman may be a better indicator of variation in shear force compared to the traditional indicators, sarcomere length, cooking loss and pHu.

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Appendix 2.

Predicting tenderness of fresh intact ovine *longissimus thoracis lumborum* using Raman Spectroscopy

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Abstract – Fresh intact lamb muscle was measured using a 671nm hand held Raman probe with a view to prediction of shear force at 1 and 5 days post mortem. Raman measurements were conducted on pairs of subsamples, aged at 1°C for 1 and 5 days respectively, from 80 samples of *longissimus thoracis lumborum* (loin) from different carcases. Each subsample was also measured for shear force (SF), cooking loss, sarcomere length and pHu. Shear force values were regressed on the Raman data using partial least squares (PLS) regression and on sarcomere length, cooking loss and pHu using ordinary least squares regression. For SF at 1 day, the root mean square error of prediction (RMSEP) was 13.16N and the correlation between predicted and observed values (R^2_{cv}) was equal to 0.09. Corresponding values for 5 day aged SF were R^2_{cv} = 0.02 and an RMSEP of 9.90N. This is the first Raman spectroscopy study to measure fresh lamb. Although prediction of SF is poor, the error of prediction is comparable with the traditional indicators. A combination approach of Raman spectra and traditional indicators gave a small reduction in the error of prediction. Further work is required to capture the potential benefits of Raman spectroscopy.

Key Words – shear force, meat quality assessment, sheep

V. INTRODUCTION

Tenderness is an important factor in the consumer acceptability of meat. Meat tenderness is determined by the contribution of connective tissue, myofibrillar structure, the links between them and the changes in structure with ageing [1, 2]. Due to the importance of tenderness, many attempts have been made to measure these changes [3]. Of the technologies that have been used, Raman spectroscopy has been highlighted as having potential as it is rapid, non-destructive, non-invasive and is insensitive to varying water contents [4]. Recent research has shown it has potential in predicting sensory traits of beef [5]; the effect of aging and cooking on pork [6]; and the relationship between spectra, shear force and cooking loss in lamb [7]. However, the application of these studies to the use of Raman spectroscopy for online meat assessment and prediction are limited as samples have been cooked, homogenized or frozen for measurement and bench top Raman devices have been used in most studies. In this study, the potential of a Raman hand held spectroscopic device to predict tenderness of fresh intact lamb is reported for the first time.

VI. MATERIALS AND METHODS

Samples of *m. longissimus thoracis lumborum* (LL) were taken from 80 lamb carcases sampled over four days (20 per day). One LL was removed from each carcase and was split into a caudal and medial section and randomized to two ageing periods (1 and 5 days) and held at 1°C. Each subsample was measured immediately following its assigned ageing period.

Raman measurements were taken using a hand held Raman probe [8]. Spectra were recorded with 70 mW of laser power and an integration time of 3 seconds. Ten Raman scans were completed on intact muscle perpendicular to the muscle fibres, with the silverskin removed. LL samples allocated to 5 days post slaughter ageing period were scanned for Raman measurements on day 1, vacuum packed and held at 1°C for 5 days. After ageing, the vacuum packs were opened and samples were allowed to 'bloom' for 2 hours before a freshly cut surface was re-scanned. The 10 Raman spectra per sample were averaged and normalized by dividing by its l_2-norm (square root of sum of squared intensities).

For shear tests, a section was weighed after scanning (mean 64 g). The samples were cooked for 35 min in plastic bags at 71°C in a water bath as previously described [9]. Samples were weighed once cooked to determine cooking loss (CL). Sarcomere length (SL) was measured using the laser diffraction method [10] and pHu was determined as previously described [11] on 1 day aged samples.

Prediction models for shear force using Raman spectra and alternative indicators (CL, SL and pHu) were fitted using Principal components analysis (PCA) and partial least square (PLS) regression analysis performed using R computer software [12]. For PLS, the optimal number of latent variables included was determined on the model having minimum root mean square error of prediction (RMSEP) based on 20 replications of 8-k fold cross validation. Simple linear regression was used for prediction based on cooking loss, sarcomere length and/or pHu.

VII. RESULTS AND DISCUSSION

Summary results for shear force, cooking loss, sarcomere length and pHu measurements are given in Table 1.

Table 1. Mean, standard deviation (SD) and range for shear force (SF; N), cooking loss (CL; %), sarcomere length (SL; µm) and pHu.

Trait	Ageing (days)	Mean	SD	Range (min, max)
SF	1	60.0	13.7	34.8 - 87.1
SF	5	38.8	9.9	20.9 - 64.6
CL	1	17.3	2.7	0.3 - 25.9
CL	5	19.1	4.3	0.2 - 28.8
SL	N/A	1.65	0.09	1.36 - 1.86
pHu	N/A	5.69	0.16	5.54 - 6.29

In Table 2, the RMSEP values for prediction of shear force at 1 and 5 days using different models are summarised.

Table 2. RMSEP for models using traditional indicators and Raman spectra to predict shear force values (N) of 1 and 5 day aged lamb loin.

Model Covariates	Shear Force (1 Day)	Shear Force (5 Day)
Cooking Loss (CL)	13.45	9.74
Sarcomere Length (SL)	12.94	10.07
pHu	13.98	10.12
CL, SL and pHu	12.82	10.03
Raman Spectra (1 day)	13.16	9.90
Raman Spectra (5 day)	N/A	9.92
Raman Spectra (1day) + CL, SL and pHu	12.46	9.78
Raman Spectra (5 day) + CL, SL and pHu	N/A	9.76

The best prediction using Raman, based on the square correlation between cross validated predictions and observed values (R^2_{cv}) was the prediction of shear force 1 day post mortem using Raman spectra measured on day 1. This gave a RMSEP of 13.16, using 2 latent vectors (Fig.1), with R^2_{cv} = 0.09.

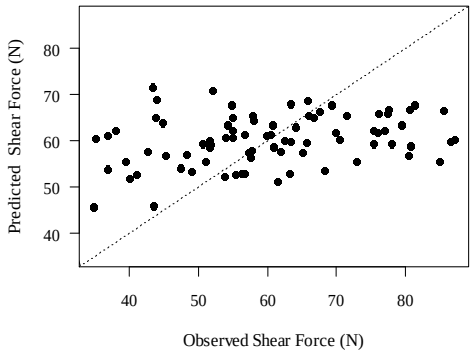

Figure 1. Prediction of shear force values at 1 day post mortem using Raman spectra analysed with 2 latent vectors.

The models for predicting shear force of 5 day aged LL with 1 or 5 day spectra, based on 2 and 1 latent vectors respectively, gave an RMSEP of approximately 9.9 with R^2_{cv} = 0.02, at best.

Of the traditional indicators, sarcomere length was a slightly better predictor of shear force (RMSEP= 12.94) than cooking loss (RMSEP= 13.45) or pHu (RMSEP= 13.98) the relationship between shear force and sarcomere length is weak (R^2 = 0.14; Fig 2) at 1 day and by day 5 there was no relationship. This highlights the significance of fibre contraction during *rigor mortis* in determining initial tenderness, while proteolysis and the subsequent degradation of the myofibrillar structure diminishes some of the influence of sarcomere length for aged meat [13].

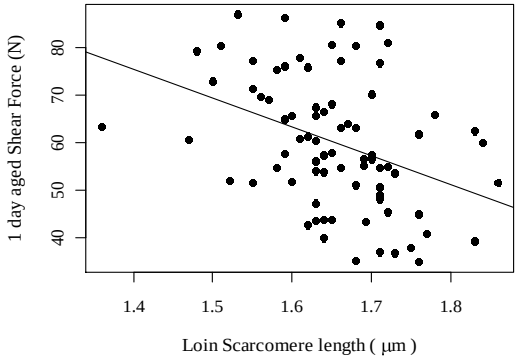

Figure 2. The relationship between shear force values (N) at 1 day post mortem and sarcomere length (µm).

Cooking loss had a weak relationship with shear force values at both 1 and 5 days post slaughter (R^2 = 0.07 and R^2 = 0.06 respectively). Less variation in the shear force values at day 5 (Table 1) contributed to the lower error at 5 days (RMSEP = 9.74) and although this is the lowest prediction error of any of the models, it is not significantly different to the prediction error of Raman spectra predictions at 5 days.

It has been established in literature that pHu also contributes to shear force [13, 14] however, data in this study shows no relationship between pHu and either 1 or 5 day shear force (P > 0.05). Although there was significant variation in shear force data (Table 1), there were no measurements in this study that would be classified as being very tender (below 27N) [7]. Combined with few samples that had a pH above 5.8, it is not surprising that pH wasn't significant predictor effect in our study.

Co-efficient of determination levels in this study are low in comparison to the previous Raman study conducted on lamb which had a co-efficient of R^2 = 0.72 [7]. It should be stressed that the one previous report with this probe was for lamb that was frozen and thawed prior to measurement. As Raman spectroscopy is sensitive to the changes that occur during freezing and thawing [15, 16], it is hypothesised that movement of cellular water and changes in ionic charges as the muscle cell structures change during freezing and thawing [17] improves the ability of Raman to predict shear force.

Previous studies using Raman spectroscopy to measure porcine *m. longissimus* and beef silverside also produced relatively high R^2_{cv} values of 0.77 and 0.75 for predicting shear force respectively [5, 6]. As the Raman signal is weak in comparison to fluorescence of biological samples, it is sensitive to spectral acquisition parameters needed to improve signal to noise ratios and the concentrations that can be quantitatively measured [18]. Acquiring several repetitions in the same position via longer accumulation times during scanning can overcome this [18]. Consequently it is expected that a 3 minute accumulation time [5] would improve prediction models in comparison to results reported here where only a 3 second accumulation was used. While 3 minutes is too long for online application, it is proposed that increasing the accumulation time by 10-12 seconds will improve the prediction.

VIII. CONCLUSION

The measurement of 80 LL lamb samples with a hand held Raman probe showed a poor ability to predict shear force based on the Raman spectra. Prediction of shear force 1 day post mortem had a slightly better accuracy in comparison to predicting shear force based on spectra measured on day 5. In comparison traditional meat science measures sarcomere length and pHu were also poor predictors of shear force. However measurement of LL on-line in lamb carcases using Raman Spectroscopy using these experimental parameters for prediction of shear force does not appear to provide the improvement in accuracy that industry would require. This conclusion though is restricted to the LL and these experimental parameters and excludes other muscles and also the measurement of other traits, such as intramuscular fat and other experimental parameters such as longer accumulation times, which are yet to be studied.

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of Matt Kerr, Tracy Lamb and Kristy Bailes (NSW DPI) who assisted in measurement of the samples.

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Appendix 1.

Proposed Experiment 2

Objectives:
1. To verify that the models developed in Experiment 1 for the topside can be used to predict shear force of 5 day aged meat.
2. To establish whether Raman measurements obtained prior to rigor mortis are useful in predicting shear force of 5 day aged meat.

Methodology:
Samples *m. semimembranosus* (SM) from 80 lambs (20 per measuring day with 4 consecutive measuring days) will be scanned online with the Raman probe. Lamb carcases will be randomly selected off the chain, being of mixed sex and consignment, of different ages and backgrounds, thus representing typical animals processed by the abattoir.

Measurements of the sample prior to rigor mortis will be taken on a fresh muscle surface *in situ* on the carcase, with the subcutaneous fat removed. It is proposed to do 10 scans/measurements perpendicular to the muscle fibre (Fig. 1) 1-2 hours after slaughter. Each spectral measurement will be completed using a 671nm laser, an integration time of 3 seconds and 8 repetitions, resulting in a single scan time of 15secs and a 7.5 min interval for total Raman spectra measurement per carcase (2.5 hours per 20).

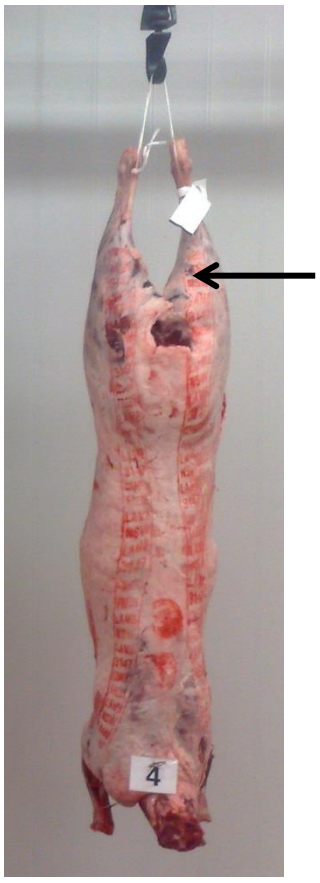

Figure 1. Lamb carcase demonstrating the position for sampling the SM with the Raman Spectroscopy Hand Held probe.

Carcases will be electrically stimulated (as per industry practice), Achilles hung and chilled as normal with the temperature and pH decline measured in the SM. At 24 hours post mortem the SM will be boned out, 10 scans will be taken perpendicular to the muscle fibre (Fig 2.), pH measured and a section removed for sarcomere length, histology and particle size analysis. The remaining SM will be vacuum packed and held at -1 to 2˚C for 4 days.

Figure 2. Measuring the SM with a Raman hand held probe, perpendicular to the muscle fibres.

After 4 days aging (5 days post slaughter) samples will be removed from packaging and allowed to 'bloom' for 2 hours prior to measurement to avoid issues with fluorescence. Once allowed to 'bloom' a further 10 scans will be taken perpendicular to the muscle fibre, with the Raman hand held probe (Fig. 2). To avoid issues with oxygen saturation a freshly cut muscle sample will be scanned.

To allow for data comparison between aging periods and to validate the model previously developed with experiment 1, all spectral data will be obtained using a 671nm laser, with an integration time of 3 seconds and no repetitions. To give an indication of how repetitions affect the model, 8 repetitions will be stored separately to the initial accumulation to enable a comparison.

At 5 days post slaughter after scanning sections will be removed for shear force, particle size analysis, pHu, collagen and histology.

Experiment 3

Objectives:

1. To establish what changes occur to Raman measurements obtained as the carcase goes into rigor mortis and achieves pHu.
2. To investigate the impact of the timing of pre-rigor measurements on 5 day shear force.

Methodology:
Samples *m. semimembranosus* (SM) from 32 lambs (8 per measuring day with 4 consecutive measuring days) will be scanned online with the Raman probe (Fig. 1 University of Bayreuth). Lamb carcases will be randomly selected off the chain, being of mixed sex and consignment, of different ages and backgrounds, thus representing typical animals processed by the abattoirs

Measurements of the sample prior to rigor mortis will be taken on a fresh muscle surface *in situ* on the carcase, with the subcutaneous fat removed. It is proposed to do 10 scans/measurements perpendicular to the muscle fibre (Fig. 2) every hour, for 4 hours, at which point it is expected the carcase will have entered rigor. Immediately after Raman measurements are taken, the SM will be measured with a pH probe.

Carcases will be electrically stimulated, Achilles hung and chilled as normal with the temperature and pH decline measured in the SM, each time a scan is measured. At 24 hours post mortem the SM will be boned out, 10 scans will be taken perpendicular to the muscle fibre (Fig 3.). A further 10 scans will be measured perpendicular to the muscle fibre but using the same fibre orientation to replicate an *in situ* measurement. Sub-samples for particle size analysis and sarcomere length will be collected and the pH will be measured with the probe again before the remaining SM will be vacuum packed and held at -1 to 2˚C for 4 days.

After 4 days aging (5 days post slaughter) samples will be removed from packaging and allowed to 'bloom' for 2 hours prior to measurement to avoid issues with fluorescence. Once allowed to 'bloom' a further 10 scans will be taken perpendicular to the muscle fibre, with the Raman hand held probe (Fig.3). To avoid issues with oxygen saturation, a freshly cut muscle sample will be scanned. At 5 days post slaughter after scanning sections will be removed for shear force, particle size analysis and pHu. All spectral data will be obtained using a 671nm laser, with an integration time of 3 seconds and 8 repetitions.