

## Verification of the production system of beef carcasses by using spectroscopic technologies on the subcutaneous fat (#218)

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### Introduction

Verifying the production system of beef in Australia is dependent on audits and reliant on producers following requirements set by processors, which vary for individual grain and grass-fed brands. Maintaining transparency through the supply chain for grass fed beef is a significant cost in the form of auditing, and an even greater potential cost if there is a failure in the auditing process as no objective verification of production system is in place. Consequently, there is opportunity to develop a carcass measure that can be used to verify beef production systems. This research tested the viability of Raman Spectroscopy (RS) to accurately differentiate between production systems. RS was chosen as the differences in the subcutaneous fat are believed to come from changes in the fatty acid and  $\alpha$ -carotene concentrations and these chemical compounds are made up predominantly by C-H, C-C and C=C bonds which RS is suited for detecting [1].

### Methods

From two abattoirs a total of 300 beef carcasses were sampled (150 grass-fed and 150 grain-fed). All diets were verified through the current supply chain methods and directly with the producers of the sampled carcasses. At 24 h post mortem Raman spectra were collected using a Mira hand-held device (Metrohm) in 3 positions on the point end brisket using an integration time of 3 s. Post scan the subcutaneous fat that was scanned was excised for fatty acids analysis using the Lepage and Roy [2] method.

The 3 spectra per carcass were averaged and the wavelengths reduced to 600–1800 $\text{cm}^{-1}$ , scaled continuum correction was then applied to correct for non-Raman background contributions. Principal components analysis (PCA) was undertaken and peaks of interest were identified numerically by taking second differences. Statistical analysis of the fatty acid composition was completed using linear mixed effects models, deriving predicted means and standard errors and calculating least significant differences between means ( $P = 0.05$ ) from the carcasses of each feed type. To account for any batch effects, day of measurement was included as a random effect, with cattle feed type as a fixed effect. All statistical analyses were completed in R Core Software using the 'emmeans' package and prospectr package.

### Results

The carcasses from grain-fed cattle show a higher intensity at the wave-

lengths 1069  $\text{cm}^{-1}$ , 1127  $\text{cm}^{-1}$ , 1301  $\text{cm}^{-1}$  and 1445  $\text{cm}^{-1}$  (Fig 1). Notably, grain-fed cattle did not have a higher intensity at 1658  $\text{cm}^{-1}$ , as this peak was highest in the carcasses from grass-fed cattle.

Saturated fatty acids (SFA) were significantly ( $P < 0.05$ ) higher in grain-fed cattle (11.1 g/100 g  $\pm$  0.43 s.e.) than grass-fed cattle (8.3 g/100 g  $\pm$  0.43 s.e.; Table 1).

Clustering evident in the PCA plot (Fig 2) indicates variation in the spectra is associated with production system (grass versus grain), with the first two principal components accounting for 93 % of data variation.

### Conclusion

Given that SFAs have no double bonds C=C and C-H bonds are the dominant features of spectra from SFAs, and they have been characterised at wavelengths of approximately 1068  $\text{cm}^{-1}$ , 1120  $\text{cm}^{-1}$ , 1301  $\text{cm}^{-1}$  and 1445  $\text{cm}^{-1}$  [3] in pork subcutaneous fat. The peak at 1658  $\text{cm}^{-1}$  in grass fed cattle is more difficult to classify and requires further investigation of the bonds reflected in this peak. Grass-fed samples showed a more intense peak at 1658  $\text{cm}^{-1}$ , which has previously been associated with an increase in the number of unsaturated fatty acids. Grass fed cattle had a lower omega-6 to omega-3 ratio, but did not differ from grain-fed cattle in terms of total polyunsaturated fatty acids. Our results indicate that fat from grass and grain-fed cattle did not significantly differ in mono- or polyunsaturated fatty acids concentrations.

The PCA shows the ability of RS to split the feeding groups based solely on the spectra obtained, and further analysis incorporating the fatty acid data may result in a clearer split of samples. Testing of this model with a more robust data set including different levels of grain and grass feeding will assist in the development and implementation of this system as an objective measurement to be used to verify production system.

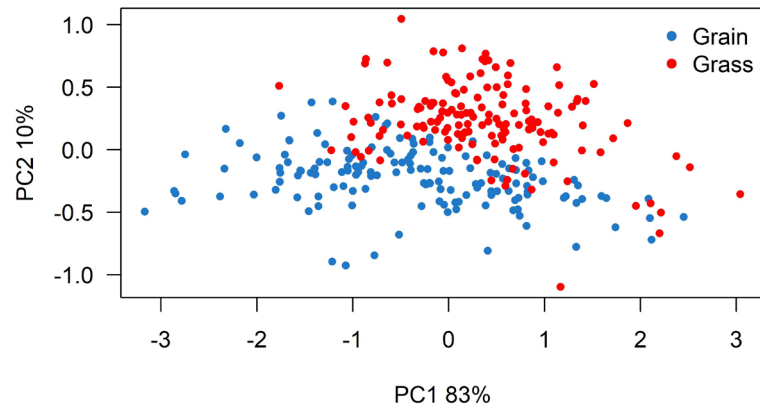
Grass-fed and grain-fed cattle are able to be successfully differentiated through the use of Raman spectroscopy. Further investigation into the cause of the bond at 1658  $\text{cm}^{-1}$  will be beneficial in understanding what in the grass fed samples is causing the difference in the RS spectra. Further research could lead to the adoption of RS as an online method of verifying beef from different production systems.

1 Yang, D. & Y. Ying (2011). Applications of Raman Spectroscopy in Agricultural Products and Food Analysis: A Review. Applied Spectroscopy Reviews,

46(7): 539-560.

2 Lepage, G. & C. C. Roy (1986). Direct transesterification of all classes of lipids in a one-step reaction. Journal of lipid research, 27(1): 114.

3 Olsen, E. F., C. Baustad, B. Egeland, E.-O. Rukke & T. Isaksson (2010). Long-term stability of a Raman instrument determining iodine value in pork adipose tissue. Meat Science, 85(1): 1-6.



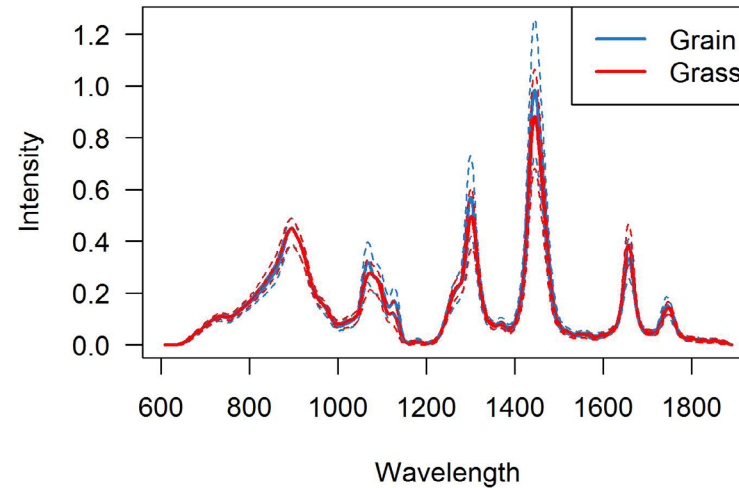
**Figure 2.** Scatter plot PCA on Raman spectra from 150 grain and 150 grass-fed beef carcasses

Scatter plot of the first two principal components (PC1 and PC2) of the principal component analysis (PCA) completed on Raman spectra collected from the subcutaneous fat from 150 grain and 150 grass-fed beef cattle carcasses.

Fatty acid	Grain-Fed		Grass-Fed	
	LSM	s.e.	LSM	s.e.
Polyunsaturated Fatty Acids	0.7	0.03	0.6	0.03
<b>Totals (g/100g)</b> Monounsaturated Fatty Acids	13.6	0.57	12.1	0.57
Saturated Fatty Acids	11.1b	0.43	8.3a	0.43

Different letters within rows indicate significance between means ( $P < 0.05$ ).

**Figure 1.** Fatty acid composition of subcutaneous fat from 150 grass-fed and 150 grain-fed carcasses Least square means (LSM) and standard errors (s.e.) of the main groups of fatty acid composition from the subcutaneous fat on carcasses of 150 grass-fed and 150 grain-fed beef cattle



**Figure 1.** Raman spectra collected from subcutaneous fat of 150 grass-fed (red) and 150 grain (blue) Mean Raman spectra collected from the subcutaneous fat of 150 grass-fed (red) and 150 grain-fed (blue) beef cattle carcasses where the dashed lines show the 5 - 95% quantiles.

## Notes