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Estimation of the age/maturity of beef and sheep using spatially resolved visible-near-infrared spectroscopy

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Abstract

Animal age and maturity have an important effect on beef and lamb carcases and meat quality. Current methods to determine age and maturity, using dentition and ossification, are not without flaws yet provide a solid description of meat quality. This description however has the potential to be improved. In this study, a fibre-optic probe-based spatially resolved spectroscopy (SRS) system was used to investigate the possibility of using visible-near-infrared (Vis-NIR) spectra for predicting the chronological and physiological age of beef cattle and carcase traits associated with meat quality.

Eighty Angus cattle, with accurate date of birth, were sampled from 3 producers at 2 abattoirs with animal background and slaughter data recorded. The hides were scanned at 4 different locations (neck, armpit, rib region and under hind leg) using the SRS system, which contains 5 concentric rings of detection fibres (centre-to-centre distances of 0.5, 1.0, 1.5, 2.0, and 2.5 mm from the source fibre) with a wavelength range of 390 – 1000 nm. Striploin and eye round muscles were collected for scanning and meat quality analysis.

Overall, data collected and analysed to date indicates that the prediction of chronological age is best determined using spectra collected on the hide at the neck location (**R^2_{CV} = 0.78, RMSECV = 1.6 years**). The results also suggest there is potential for predicting shear force when spectra from eye rounds are used (**R^2_{CV} = 0.69, RMSECV = 5.7 N**) as well as soluble collagen content (**R^2_{CV} = 0.73, RMSECV = 0.44**). Analysis also indicated that striploin muscle scans have the potential to predict MSA marbling scores when scans of samples, at 2 days post mortem, were used (**R^2_{CV} = 0.49, RMSECV = 85**). The prediction of physiological age through calibration models built using scans of hides to predict ossification scores was considered. Analysis suggests that the predictive ability of the scans is limited (**R^2_{CV} = 0.63 and RMSECV = 101**). This appears to be due to the lack of sensitivity of ossification scores for cattle over the age of 5-6 years. Similar results were obtained when muscle scans at 2 days post mortem were used with eye round muscle scans performing slightly better (**R^2_{CV} = 0.62 and RMSECV = 103**) than the strip loin muscle scans (**R^2_{CV} = 0.55 and RMSECV = 112**).

Additional data from cattle, uniformly covering from 0 to approximately 12 years old, is required in order to ensure that robust models are built to prevent model overfitting that can arise when internal cross validation is used along with wavelength selection. This additional data will allow for testing using an external validation data set.

Executive summary

Due to the relationships between animal age and maturity, collagen structure and tenderness, “age” is a critical factor in determining the market suitability of carcases and eating quality within the Meat Standards Australia (MSA) grading system. Consequently, demarcation occurs after 30 months with older carcases attracting discounts, yet considerable debate still remains on the best way to determine “age”. Despite cattle processors assessing the changes of cartilage along the spine by ‘ossification’, as an additional tool to determine physiological age, estimates can vary with nutrition and health stress. Consequently, an inexpensive, accurate and standardised measure of age could be utilised by industry to facilitate better identification of the true chronological and physiological age of animals.

The main aim of this project was to examine whether visible-near-infrared (Vis-NIR) spectra of hide samples could be used to accurately estimate the age/maturity of slaughter animals, with a particular focus on beef. Another aim was to investigate the possibility of predicting meat quality with direct relevance to the estimation of shear force and soluble collagen. Due to the encouraging results obtained from the muscle spectra data, the potential of the instrument to predict marbling scores using Vis-NIR scans of muscle tissue was also undertaken.

This work used a device which provided spatially resolved spectroscopy (SRS) measurements instead of standard reflectance spectroscopy measurements. Spatially resolved spectroscopy consists of using a fibre-optic probe which delivers light through a fibre and collects reflected light. The reflected light is from different distances from the source fibre using detection fibres placed at different distances from the source fibre. Thus, it provides reflected signals which have traversed different paths and distances within the tissue sample. This set up was used since it provides the means to find the optimal source-detector distance in terms of calibration model performance. This information can then be used to simplify the design of the fibre probe and make the device cheaper for commercial use. The probe used in this study consisted of a source fibre which delivers the light to the sample surround by 5 concentric rings of detection fibres. These fibres collect light reflected from the sample at different radial distances from the source fibre.

Eighty Angus cattle, with accurate date of birth, were sampled from 3 producers at 2 abattoirs with animal background and slaughter data (including dentition and ossification score) were recorded. After the hides had been removed from the carcase, 4 different locations were scanned (neck, armpit, rib region and under hind leg) using the SRS Vis-NIR system with a wavelength range of 390– 1000 nm. At 24 hours post mortem (pm), the striploin and eye round muscles were collected for scanning and meat quality analysis.

Partial least squares regression was then conducted to determine the potential to predict age and meat quality traits (shear force and soluble collagen) using spectra collected from the hide and from the striploin and eye round muscle scanned at 2, 14 and 28 days pm. Internal cross validation was done using the leave-one-out method and spectra were pre-processed using an Automatic Whittaker Filter for baseline correction and mean centred. The Automatic Whittaker was chosen after comparison of model performance using a number of standard spectra pre-processing techniques using the initial set of data collected for building calibration models. Models were also built using Genetic Algorithm for wavelength selection and the performances compared with those built without wavelength selection.

Overall, data collected and analysed to date indicates that the prediction of chronological age is possible using spectra collected on the hide at the neck by ring 5 (**R^2_{CV} = 0.75, RMSECV = 1.6 years**). Although, not as accurate, the prediction using spectra collected at location C (rib region) ring 5 (**R^2_{CV} = 0.69, RMSECV = 1.8 years**) and location A (neck) ring 1 also yielded some predictive ability (**R^2_{CV} = 0.70, RMSECV = 1.7 years**). There was also some potential to predict age using the eye round (**R^2_{CV} = 0.69, RMSECV = 1.78 years**) by ring 5.

Partial least squares (PLS) regression analysis was conducted to determine the potential to predict tenderness of the eye round muscle at 2, 14 and 28 days post mortem using SRS Vis-NIR spectra. collected at 2, 14 and 28 days post mortem, respectively. Regression models were built to predict shear force values. The results suggest that there is potential for predicting shear force when spectra from the eye round are used (**R^2_{CV} = 0.69, RMSECV = 5.7 N**). When spectra from the striploin muscle were used, the predictability was generally poor. Models built to predict shear force from hide scans indicated some predictive ability particularly when scans from location C (rib region) collected by ring 5 is used (**R^2_{CV} = 0.52, RMSECV = 6.2 N**).

As in the case of shear force prediction, scans of eye round muscles indicated the ability to predict soluble collagen content (**R^2_{CV} = 0.73, RMSECV = 0.44**) while scans of striploin muscles did not indicate any ability to predict soluble collagen content. The prediction of the shear force values appears to be an indirect prediction of soluble collagen, which may explain the differences in prediction of shear force between the striploin and eye round as connective tissue plays a major role in determining meat toughness in the eye round.

Analysis also indicated that scans of striploin muscles have the potential to predict MSA marble scores when scans of samples at 2 days pm were used (**R^2_{CV} = 0.49, RMSECV = 85**).

The prediction of physiological age through calibration models built using scans of hides to predict ossification scores was considered. Analysis suggests that the predictive ability of the scans is limited with ring 5 measurements at location A providing the best results (**R^2_{CV} = 0.63 and RMSECV = 101**). This limitation is probably due to the lack of sensitivity of ossification scores for cattle over the age of 5-6 years. Similar results were obtained when muscle scans were used with eye round muscle scans performing slightly better (**R^2_{CV} = 0.62 and RMSECV = 103**) than the strip loin muscle scans (**R^2_{CV} = 0.55 and RMSECV = 112**).

While the results discussed in this report were based on models which incorporated wavelength selection by genetic algorithm, analyses were also carried out by building models without wavelength selection. In all cases, the models arising from application of wavelength selection led to lower root mean square error of cross validation. Given that the age distribution is skewed towards the age of 2-3 years with relatively lower numbers of samples across other ages, there is potential for model overfitting to occur when applying the genetic algorithm. This could lead to more optimistic estimates of predictive ability than warranted. Therefore, it is essential to collect additional data across the age span of 0 – 13 years with an approximate uniform distribution of ages. Such a dataset will allow a more robust calibration model to be built. Additionally, a sufficient number of samples which span the desired age range can be set aside to be used as an external validation (i.e. an independent test) set so that a reliable estimate of predictive ability of the models can be obtained.

Given that the commercial age for slaughter of beef in Australia is around 18-30 months old there was a delay in sampling the other age categories, mainly younger than one year old and older than 5-6 years old. Thus, data collection of vealers and cull cows is required to provide a wider distribution in age to create a more accurate and robust calibration model. It is crucial to have enough data for animals older than 5-6 years old as it has been established that eating quality differs within this age category while ossification is not entirely distinctive for animals in this age category (reaching maximum score of 590).

Analysis indicates that, in most cases, spectra from ring 5 lead to models that were better compared to measurements from other rings. This raises the possibility that, in the future it may be possible to simplify the probe design and thus reduce the cost of a commercial device for estimating age and meat quality traits in addition to possibly reducing measurement time which currently takes approximately 1 minute. Also, based on the analyses carried out, the number of locations from which data will be collected in the future can be restricted to locations A (neck) and C (rib region).

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1 Background

Animal age and maturity have an important effect on carcase and meat quality (Duarte et al., 2011; Schönfeldt et al., 2011). There is considerable debate in the red meat industry over the best way to determine the “age” of animals. It is known in sheep that teeth eruption, which is used as the “ageing” method, is often weakly related to the chronological age (Hopkins et al., 2007). As such the time span for sheep to transition from lamb to hogget based on teeth eruption can be up to 6 months. In beef, dentition and/or ossification are used. In this regard, ossification is currently the “gold standard” to estimate the maturity of animals, however this evaluation requires trained graders and as such is susceptible to human error. In addition, commercial beef cattle are mostly slaughtered around 18 to 30 months of age and at this stage the animal may have 0, 2, 4 or 6 permanent incisors. This inconsistency causes penalties in the carcase price up to 7.5%.

In the same way, carcase ossification is also strongly affected by several factors, such as nutrition, growth path, gender, genetics, hormone status and animal health. The ossification score of heifers has been observed to be15 % higher than steers, and cattle implanted with hormonal growth promotants (HGPs) have a 10% higher bone ossification (Cafe et al., 2010). The highest ossification score is 590 on the Meat Standards Australia (MSA) maturity system which is usually reached when the animal is 5 to 6 years old, yet animals are slaughtered for meat at ages up to 14 years old. Hence cattle between 6 and 14 years of age cannot be differentiated in age or physiological maturity, thus are classified and sold as the same even though data shows the eating quality of these animals is different (Bonny et al 2016). Therefore there is great potential for the current methods for determining dentition and ossification to be improved, especially for animals under 36 months and/or over 6 years of age.

In addition, dentition and ossification score show disagreement in the maturity and age classification of young cattle, which is not suitable for an industry wanting to perfectly predict the eating quality of meat. Lawrence et al. (2001) compared the US Department of Agriculture (USDA) ossification system with dentition system and showed that over 9 % of the steers were classified as maturity B (30-42 months old) but had 0 permanent incisors (typically < 18 months old). In sheep, there is no method being used to estimate maturity of the animal in the abattoir and only dentition is used to estimate age.

The variation and inconsistency of dentition and ossification scores can create significant differences in carcase price/value. Work in beef has shown that the weight of the eye lens is strongly related to true age (Raines et al., 2008), but the practicalities of adopting this measure prevent application. The use of spectroscopic technology can effectively provide information on the structure of tissues, as affected by age. Work on human’s shows that it is possible to determine the age of people using NIRS scans of skin (Ruchti et al. US Patent, 2002). In principle this should translate to animals. Interestingly, regarding the NIRS evaluation of the skin of slaughter animals, a recent study has shown NIRS of the ear skin can be a fast method to classify pork carcases on fatness and fatty acid composition (Prieto et al., 2015).

This project examines whether visible-near-Infrared (Vis-NIR) spectra of skin/hide samples can be used to accurately estimate the age/maturity of slaughter animals with particular focus on beef. Conceptually, a small area of skin and muscle will be evaluated on line and at line speed, attempting to provide an accurate estimate of animal age/maturity and its effect on eating quality.

Given that higher accuracy of estimating the age would be required in practice than shown for human skin in the literature, the proposed work will use spatially resolved spectroscopy (SRS) measurements instead of standard reflectance spectroscopy measurements. SRS consists of using fibre-optic probes which delivers light through a fibre and collects reflected light. The reflected light is sourced from different distances from the source fibre using detection fibres placed at different distances from the source fibre (Hjalmarsson and Thennadil, 2008). Thus, it provides reflected signals which have traversed different paths and distances within the tissue sample. Such as set up will provide the means to find the optimal source-detector distance in terms of calibration model performance.

2 Project objectives

(1) Evaluation of proof-of-concept Vis-NIR spectroscopy for estimating age of animals that can be used by the red meat industry.

(2) Development of a spatially resolved fibre probe spectroscopy system which includes a novel fibre-probe for non-invasive measurements of animal skin tissue being developed.

(3) Algorithms and software required for converting the measurements into an estimation of age will be developed. This will be done through an investigation of state-of-the-art methods that will be suitably modified for the purpose of estimating age in animals.

(4) An evaluation of the benefits of using spatially resolved Vis-NIR compared to standard Vis-NIRS to quantify textural characteristics (tenderness) of meat.

3 Methodology

3.1 Animal Background

Animal background, accurate date of birth, carcase and meat quality information was obtained from 80 Angus cattle from 3 producers slaughtered at 2 different abattoirs in New South Wales and Queensland. The age distribution of the 80 animals is shown in Figure 1. Accurate date of birth, breed and gender information was provided by the producers whilst dentition, ossification and marble scores were collected at the abattoirs.

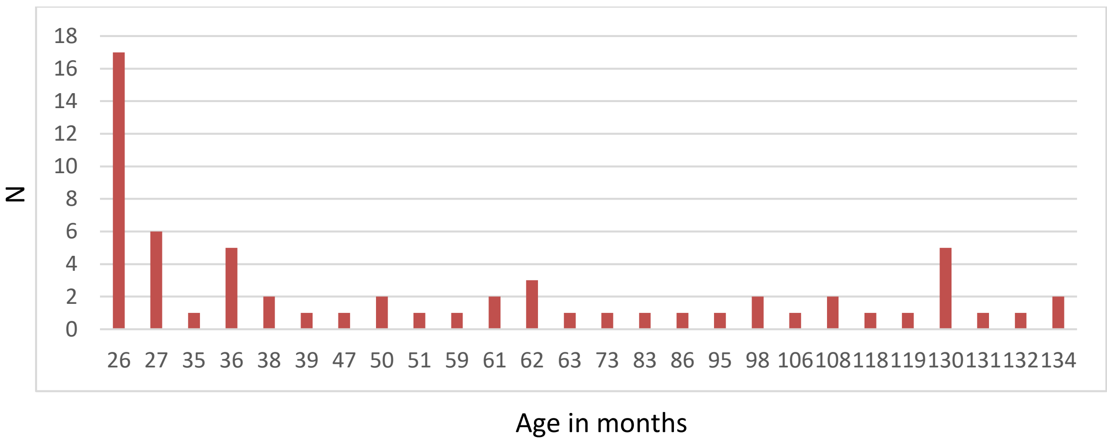

Figure 1. Chronological age (months) distribution of 80 Angus cattle with accurate date of birth.

3.2 Slaughter data collection

On the slaughter floor the hides were numbered for identification and management number was recorded, to match slaughter data with animal background data. Dentition was provided by the abattoir and a picture of each animal's mouth was taken for subsequent assessment and comparison of tooth eruption. This was in order to estimate animal age by whether they had 0, 2, 4, 6 or 8 permanent incisors.

On the day of slaughter spectra of the hides, separated from the carcase, were collected on the external surface of the hide. The collection occurred after hair was removed using clippers and metal safety razors from 4 areas measured on the left side of the hide (Figure 2). To ensure consistent SRS spectra, the measurements were undertaken with the hide sample placed on a single piece of muscle. After the scanning, hide thickness was recorded using a calliper.

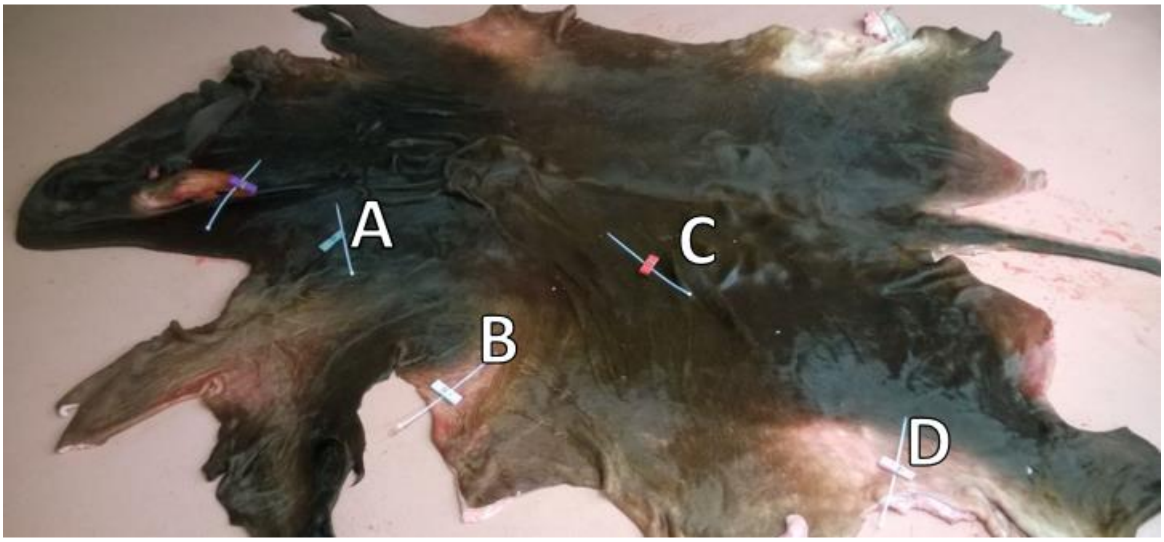

Figure 2. Hide sampling location for scanning using a spatially resolved spectroscopy. (A) Neck, (B) Armpit (C) Rib region and (D) under hind leg.

3.3 Carcase data collection

At 1 day post mortem (pm), ossification and marble scores were assessed by graders accredited by AUS-MEAT. The ossification scale ranges between 100 and 590 in increments of 10 points, as per normal abattoir processes where the sacral, lumbar and thoracic vertebrae are assessed for their calcification to estimate the physiological maturity of beef (AUS-MEAT, 2011).

The M. longissimus lumborum (striploin) and M. *semitendinosus* (eye round) were removed from each left side for objective measurement of meat quality and further Vis-NIR SRS scanning. The cuts were identified, vacuum packed in the abattoir, then placed in foam boxes and transported to the University of New England meat science laboratory.

At 2 days pm, the eye round and striploin samples were sub-sectioned into 3 portions per muscle. The portions were randomly allocated to three ageing periods, 2, 14 and 28 days. Samples allocated to the 14 and 28 days ageing treatment were vacuum packed and kept at 2-4 ^{0}C during the respective ageing period. Samples allocated to the 2 days ageing treatment were sampled for Vis-NIR SRS spectra, shear force, compression, connective tissue, sarcomere length and proteolysis analysis.

Blocks of meat, with at least 3 cm of thickness, were sampled for immediate Vis-NIR SRS measurement. Vis-NIR SRS measurements were taken with the instrument probe placed parallel to the meat fibre orientation.

Shear force was performed according to Perry, Shorthose, Ferguson, and Thompson (2001) using a Lloyd Instruments LRX Materials Testing Machine fitted with a 500 N load cell. For shear force analysis 6 subsamples, with a rectangular cross section of 15 mm wide by 6.66 mm deep (1 cm^2) , were cut from each sample, with the fibre orientation parallel to the long axis, and at right angles to the shearing surface. The mean peak force (N) required to shear through the clamped subsamples with a straight 0.64 mm thick blade pulled upward at a speed of 100 mm/min was recorded.

The method for the determination of total and soluble collagen was based on AOAC method 990.26 (AOAC, 2000), as described in detail by Starkey, Geesink, Oddy, and Hopkins (2015). Total collagen was determined in duplicate on 0.10 g samples of freeze-dried muscle powder and soluble collagen on 1.5 g of freeze-dried muscle powder by determining the hydroxyproline content. Total collagen content was calculated as hydroxyproline content × 7.25 / 1000 / (sample weight / 250) and soluble collagen as hydroxyproline content × 7.25 / 1000 / (sample weight / 400). The results were expressed in mg/g dry matter.

At 14 and 28 days pm the meat portions assigned to ageing were unpacked and sampled as described for the 2 days pm treatment for Vis-NIR SRS scanning, shear force and proteolysis analysis.

3.4 Instrument settings

The SRS device contains a probe that consists of a source fibre, which delivers light to the sample and 5 concentric rings of detection fibres to collect light reflected from the sample. Each ring consists of 4 detection fibres as illustrated in Figure 3. The light collected by different rings of fibres travels different distances within the sample and thus has potentially different levels of information. The scan setting for the SRS instrument was set to the wavelength range 380 – 1000 nm with three scans taken at each location. The three scans at each location were taken automatically with the internal setting from the device using the “Step and Glue” function. The instrument parameters were chosen based on investigation of scans from an initial set of hide samples collected from an abattoir located in the Northern Territory. The new settings for the device improved the scanning time dramatically from more than 3 minutes per location to around 1 minute per location. Two different probe designs were evaluated using the samples from the abattoir in the Northern Territory. The probe chosen for the study consisted of rings of fibres placed at centre-to-centre distances of 0.5, 1.0, 1.5, 2.0, and 2.5 mm from the source fibre respectively.

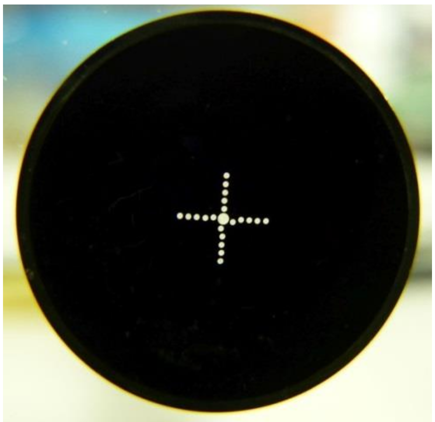

Figure 3. Source and detector fibre configuration in Probe #1. Fibre in the centre is the 400µm diameter source fibre. The surrounding fibres are the 200 µm detection fibres.

3.5 Data analysis

Noise in the spectrum has the potential to affect the analysis and the prediction of age. Visual examination indicated spikes in scans in addition to noise. To reduce noise and eliminate spikes, filtering using a combination of Savitzky-Golay Filters and spike detection was used.

Partial least squares (PLS) regression was then conducted to determine the potential to predict age and the meat quality traits using spectra collected from the hide and from striploin and eye round muscle samples scanned at 2, 14 and 28 days pm. Internal cross validation was done using the leave-one-out method and spectra were pre-processed using an Automatic Whittaker Filter for baseline correction and mean centred. The Automatic Whittaker was chosen after comparison of model performance using a number of standard spectra pre-processing techniques using the initial set of data collected for building calibration models. The Genetic Algorithm (GA) was also used to selection relevant wavelengths and the performances compared with those built without wavelength selection.

The optimal number of latent variables for each model was determined by the reduction in root mean square error of cross validation (RMSECV), where the number of latent variables used was the lowest number of latent variables with a significant (greater than 20%) reduction in the RMSECV compared to the null model (prediction of the trait using only the average of the meat quality trait of interest). Model building and analysis were carried out using Matlab (The Mathworks Inc. (2016), MATLAB, 64-bit (win64)) and the PLS Toolbox (Eigenvector Research, 2016).

In this study analyses were carried out to investigate the following:

(1) Ability of Vis-NIR reflectance scans of hides to predict chronological and physiological age of cattle.

(2) Impact of measurement location on the estimation of age of cattle. This investigation was carried out to find the best measurement location to acquire spectra from hide to estimate age. Four locations were chosen for this study (see Figure 2), and model performance compared to decide on the best location for this purpose.

(3) Ability of eye round and striploin muscle scans to predict chronological and physiological age.

(4) Ability of eye round and striploin muscle scans to predict shear force.

(5) Ability of eye round and striploin muscle scans to predict soluble collagen content.

(6) Ability of spectra acquired from hide samples to predict shear force.

(7) Ability of spectra acquired from striploin muscle to predict MSA marbling scores. The impact of ageing meat on model performance was investigated.

(8) The potential of wavelength selection using genetic algorithm (GA) to improve model performance.

(9) The optimum source – detector distance from which scans should be acquired for building the model for estimating age and meat quality traits.

4 Results and discussion

4.1 Meat quality traits

Summary statistics of animal age, maturity and meat quality traits of beef striploin and eye round muscle samples (Table 1) highlight the bias present in the animal ages of cattle sampled for this study and the skew in the dataset towards cattle between 2 – 3 years of age. This is within the industry standard slaughter age for commercial cattle in Australia. However, it is not representative of the entire slaughter. Furthermore, Table 1 also highlights the difference in meat quality traits between the eye round and striploin muscles, which is unsurprising given that the eye round is a locomotive muscle and therefore requires a greater amount of connective tissue to support the movement of the leg.

Table 1. Summary statistics for a range of animal age/maturity and meat quality traits of beef striploin and eye round.

Variable	Mean	SD	Minimum	Maximum
Age (years)	4.5	3.2	2.0	11.8
Dentition	4	-	4	8
Ossification score	290	-	140	590
Striploin				
Shear force 2 days (N)	50.8	13.8	30.0	105.3
Shear force 14 days (N)	37.5	8.4	19.0	61.6
Shear force 28 days (N)	36.7	8.5	23.3	63.4
Total collagen (mg/g dry matter)	15.0	3.6	8.1	28.2
Soluble collagen (mg/g dry matter)	1.7	0.7	0.7	4.7
Soluble collagen (%)	11.5	4.2	4.2	25.3
Eye Round				
Shear force 2 days (N)	59.2	8.9	42.7	80.4
Shear force 14 days (N)	56.3	6.5	38.9	74.2
Shear force 28 days (N)	56.1	7.1	37.7	75.5
Total collagen (mg/g dry matter)	25.1	4.5	13.3	36.0
Soluble collagen (mg/g dry matter)	2.1	0.9	0.8	4.6
Soluble collagen (%)	8.4	2.7	2.8	16.0

4.2 Prediction of chronological age and ossification

4.2.1 Prediction of chronological age using Vis-NIR scans of hide

Analysis of the spectra indicates that there is potential to predict the chronological age of beef cattle by using the Vis-NIR SRS as shown in Table 2. These results suggest that measurements at location A (neck) tend to have consistently better model performance across all rings with the lowest value obtained when spectra measured by ring 5 is used to predict chronological age (R^2_{CV} = 0.75, RMSECV = 1.6 years; Fig 4). Also, across the different locations, models using spectra from ring 5 tend to be consistently better. Ring 5 at location B (armpit) (R^2_{CV} = 0.78, RMSECV = 1.5 years) and, to a lesser extent, ring 5 location C (rib region) (R^2_{CV} = 0.69, RMSECV = 1.8 years) also have the potential to predict age.

Table 2. Performance of models to predict chronological age using scans of the hides. Models were built using hide scans from the 4 specified locations (A =neck, B= armpit, C = rib region and D = under hind leg) and measurements from each of the 5 rings of detection fibres.

Location	Ring	RMSECV (years)	R^2_{CV}	LV
A	1	1.7	0.70	4
A	2	2.0	0.63	6
A	3	1.8	0.68	7
A	4	1.9	0.64	6
A	5	1.6	0.75	7
B	1	1.7	0.71	7
B	2	2.4	0.46	3
B	3	2.2	0.53	7
B	4	2.1	0.55	4
B	5	1.5	0.78	6
C	1	2.0	0.62	5
C	2	2.1	0.59	5
C	3	2.2	0.54	4
C	4	2.1	0.55	4
C	5	1.8	0.69	6
D	1	2.3	0.50	3
D	2	2.0	0.59	5
D	3	2.3	0.49	4
D	4	2.3	0.49	4
D	5	2.4	0.44	3

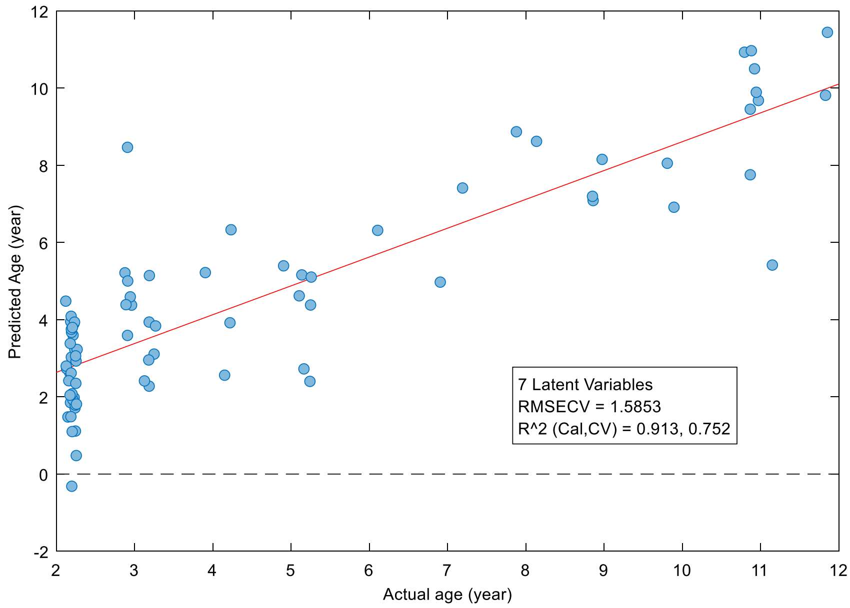

Figure 4. Prediction of age at location A (neck) using spectra collected by ring 5 (R^2_{CV} = 0.75, RMSECV = 1.6 years).

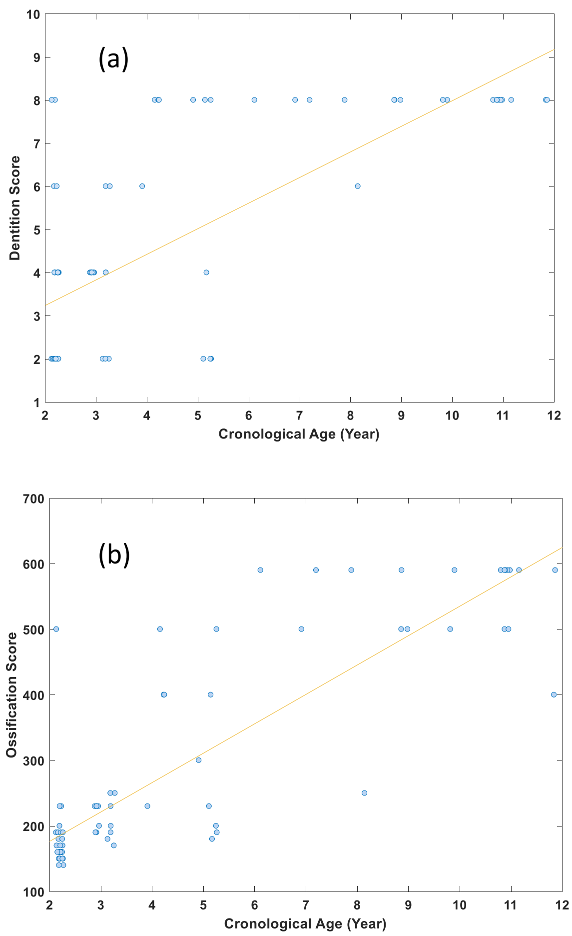

Figure 5. Correlation of chronological age with (a) dentition and (b) ossification score.

The predictive error for all of these models is likely to be reduced if further animals are included in the calibration model so that the frequency of ages is more evenly spread across measurement categories. It should also be noted that the use of GA for wavelength selection, when using a dataset which does not have a uniform age spread and a relatively small number of samples, could result in overfitting leading to more optimistic estimates of the prediction error. Furthermore, it has been established that eating quality differs within older age categories (e.g. cull cows over 5 years old) however ossification is not distinctive for animals in this physiological age category (Figure 5b). It is crucial to have enough data for animals older than 5-6 years old in a wide spread up to around 13 years old in order to build robust calibration models and to reduce the chances of overfitting.

4.2.2 Prediction of chronological age using Vis-NIR scans of muscle tissue

Spectra collected from scanning the eye round muscle could predict age with R^2_{CV} values of over 0.65 (Table 4). Spectra from striploin muscle samples indicated limited ability to predict age. Furthermore, the error when muscle samples are used is higher than those obtained when hide samples were used.

Table 3. Performance of models using striploin and eye round spectra at 2 days pm to predict chronological age.

Muscle	Ring	RMSECV (years)	LV	R^2_{CV}
	1	2.38	4	0.45
	2	2.42	4	0.44
Striploin	3	2.39	4	0.44
	4	2.36	5	0.47
	5	2.50	4	0.39
	1	1.81	5	0.68
	2	1.80	6	0.68
Eye Round	3	1.93	5	0.64
	4	1.84	4	0.67
	5	1.78	5	0.69

4.2.3 Prediction of ossification using Vis-NIR scans of hide

Since ossification score is not sensitive to age beyond 5-6 years, reaching a maximum score of 590, calibration models were built in two ways. A model for predicting ossification scores from Vis-NIR scans of hide was built using the entire dataset. This included cattle with ages above 6 years old for which the ossification scores were at the maximum of 590. Another model was built using samples from cattle with ossification score of less than 580. Table 4 shows the performance of the models for these two cases. When all the samples are used, measurements from ring 5 at location A gives the best results (R^2_{CV} = 0.63 and RMSECV = 101). However, the high error indicates that the predictability of ossification scores using scans of hides is limited. Figure 6(a) shows the corresponding predicted ossification score vs. the MSA score. A large uncertainty in the predicted scores can be observed at the higher end of the scale which is probably due to the lack of sensitivity of the ossification scores to the age of cattle beyond 5 – 6 years of age. Reducing the range of the ossification scores so that cattle over the age of 5 years old had little impact (R^2_{CV} = 0.45 and RMSECV = 88) with a reduction in error which can be attributed to the removal of the ossification score of 590 which includes cattle above the age of 6 years rather than an improvement in the predictability as can be seen by comparing Figure 6(a) and (b). This would explain the lower R^2_{CV} for this case.

Table 4. Performance of models using spectra of hides to predict ossification scores

Skin Location	Ring	All Samples			Samples with ossification score less than 580		
		RMSECV	LV	R^2_{CV}	RMSECV	LV	R^2_{CV}
A	1	105	4	0.60	98	4	0.32
	2	106	4	0.59	94	4	0.37
	3	116	4	0.51	103	3	0.24
	4	115	4	0.52	94	3	0.38
	5	101	5	0.63	88	5	0.45
B	1	128	5	0.42	107	5	0.22
	2	129	5	0.40	107	5	0.23
	3	123	5	0.45	109	5	0.19
	4	110	7	0.56	106	5	0.22
	5	118	5	0.50	98	5	0.33
C	1	117	7	0.51	107	3	0.18
	2	140	4	0.29	108	4	0.18
	3	136	6	0.36	105	5	0.23
	4	135	4	0.33	105	3	0.21
	5	136	4	0.33	104	4	0.19
D	1	134	6	0.35	118	4	0.07
	2	126	5	0.43	107	8	0.24
	3	129	6	0.40	110	6	0.17
	4	134	6	0.36	109	5	0.17
	5	134	5	0.35	114	1	0.07

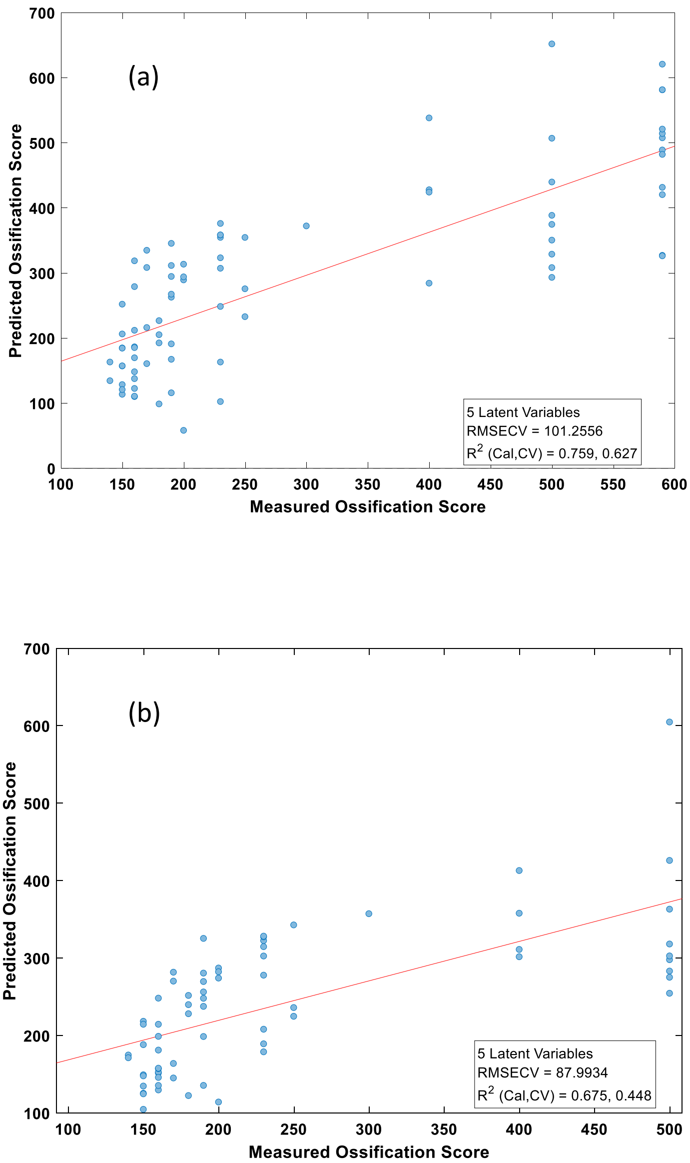

Figure 6. Prediction of MSA ossification score using spectra collected by ring 5 at location A, (a) using all samples and (b) using samples with ossification score less than 580.

4.2.4 Prediction of ossification using Vis-NIR scans of muscle tissue

Table 5 summarises the performance of models to predict ossification by using spectra from strip loin and eye round muscle at 2 days post mortem. As in the previous section, models were built using all the samples in the dataset as well as by restricting the dataset to samples with ossification score less than 580. The results suggest that, in general, the eye round muscle scans have slightly better predictive ability compared to strip loin muscle scans. When all the samples are considered, the best performance for strip loin muscle is obtained when ring 4 is used (R^2_{CV} = 0.55 and RMSECV = 112). For eye round muscle, the best performance is obtained when ring 1 is used (R^2_{CV} = 0.62 and RMSECV = 103).

Table 5. Performance of models for predicting ossification scores using spectra from strip loin and eye round muscle.

		All samples			Samples with ossification score of less than 580		
Muscle	**Ring**	**RMSECV**	**LV**	**R^2_{CV}**	**RMSECV**	**LV**	**R^2_{CV}**
Strip Loin	1	122	4	0.47	100	4	0.31
	2	119	5	0.49	93	5	0.38
	3	127	4	0.41	99	4	0.31
	4	112	6	0.55	101	6	0.31
	5	125	4	0.43	103	5	0.27
Eye Round	1	103	6	0.62	87	6	0.46
	2	115	6	0.52	92	7	0.41
	3	125	4	0.44	95	5	0.37
	4	106	5	0.59	91	6	0.42
	5	106	6	0.60	94	4	0.37

As was the case when scans of hide were used to predict ossification scores, the error is high and indicates limited predictive ability of the muscle scans despite the R^2_{CV} values suggesting that the model is statistically significant. Limiting the ossification scores to less than 580 also shows the same behaviour as was seen for hide scans, namely, a reduction in R^2_{CV}.

Table 6. Performance of models to predict shear force at 2, 14 and 28 days pm using eye round spectra at 2, 14 and 28 days pm, respectively.

Ageing period	**Ring**	**RMSECV**	**LV**	**R^2_{CV}**
2 days	1	6.44	6	0.48
	2	7.09	6	0.37
	3	8.31	4	0.18
	4	7.20	5	0.37
	5	5.70	7	0.58
14 days	1	6.90	5	0.41
	2	6.98	4	0.39
	3	6.96	4	0.38
	4	6.86	6	0.41
	5	7.35	4	0.32
28 days	1	6.65	5	0.46
	2	6.44	5	0.48
	3	6.81	5	0.42
	4	6.61	5	0.45
	5	5.83	7	0.57

4.3 Prediction of meat quality traits

4.3.1 Prediction of shear force using scans of eye round muscle

Table 6 summarises the performance of models built to predict shear force from eye round spectra Models from all 3 ageing periods (2, 14 and 28 days pm). Results indicated the ability to predict shear force from eye round spectra with ring 5 spectra at 2 days pm demonstrating the best performance (R^2_{CV} = 0.58, RMSECV = 5.70).

4.3.2 Prediction of shear force using scans of striploin muscle

Table 7 summarises the performance of models built to predict shear force from striploin spectra at 2, 14 and 28 days pm, with wavelength selection using GA. It was found that models were not able to predict shear force except for an anomalous result for ring 5 (R^2_{CV} = 0.43, RMSECV = 10.3 and). It is not clear why this model would show a high R^2_{CV} value which is appreciably different from results when other rings are used. However, given the high RMSECV, this result should be taken to indicate unsatisfactory ability to predict shear force.

Table 7. Performance of models to predict shear force at 2, 14 and 28 days pm using striploin spectra at 2, 14 and 28 days pm, respectively.

Ageing Period	Ring	RMSECV	LV	R^2_{CV}
2 days	1	13.76	2	0.009
	2	13.85	2	0.005
	3	13.91	2	0.003
	4	13.85	2	0.004
	5	10.31	7	0.43
14 days	1	8.25	2	0.03
	2	8.26	2	0.03
	3	8.30	1	0.02
	4	8.24	1	0.03
	5	7.54	4	0.20
28 days	1	8.49	1	0.04
	2	8.49	1	0.00
	3	8.51	1	0.00
	4	8.48	1	0.01
	5	8.51	1	0.00

4.3.3 Prediction of shear force using hide scans from different locations

Table 8 summarises the performance of models to predict shear force using spectra of the hide samples at the four locations and using the scans acquired by each of the 5 rings. Shear force measurement of the eye round at 2 days post mortem were used as a response variable to build the prediction model using hide samples spectra. The results show some potential for measuring shear force from hide samples when wavelength selection is used as part of the calibration. Location C ring 5 provided the best performance (R^2_{CV} = 0.52, RMSECV = 6.18) compared to the other locations and rings. In general, the R^2_{CV} based on different locations were less than 0.5. Location A (neck), which consistently provided better-performing models for estimating age has in this case led to models with the lowest R^2_{CV}.

Figure 8 depicts the prediction of shear force measurement of eye round muscle samples at 2 days pm using spectra from hide samples from location C (rib region).

Table 8. Prediction of shear force measurement of eye round using scans of hide at 4 specified locations (A =neck, B= armpit, C = rib region and D = under hind leg).

Location	Ring	RMSECV (N)	LV	R^2_{CV}
A	1	7.66	2	0.26
A	2	7.62	2	0.27
A	3	7.75	2	0.24
A	4	7.57	2	0.28
A	5	7.65	2	0.26
B	1	7.77	4	0.24
B	2	7.41	5	0.34
B	3	6.43	7	0.49
B	4	7.24	6	0.35
B	5	7.51	4	0.29
C	1	7.15	6	0.37
C	2	7.64	7	0.31
C	3	7.33	5	0.33
C	4	7.13	5	0.37
C	5	6.18	4	0.52
D	1	7.83	3	0.23
D	2	7.62	5	0.28
D	3	7.44	5	0.31
D	4	7.79	3	0.24
D	5	7.67	5	0.27

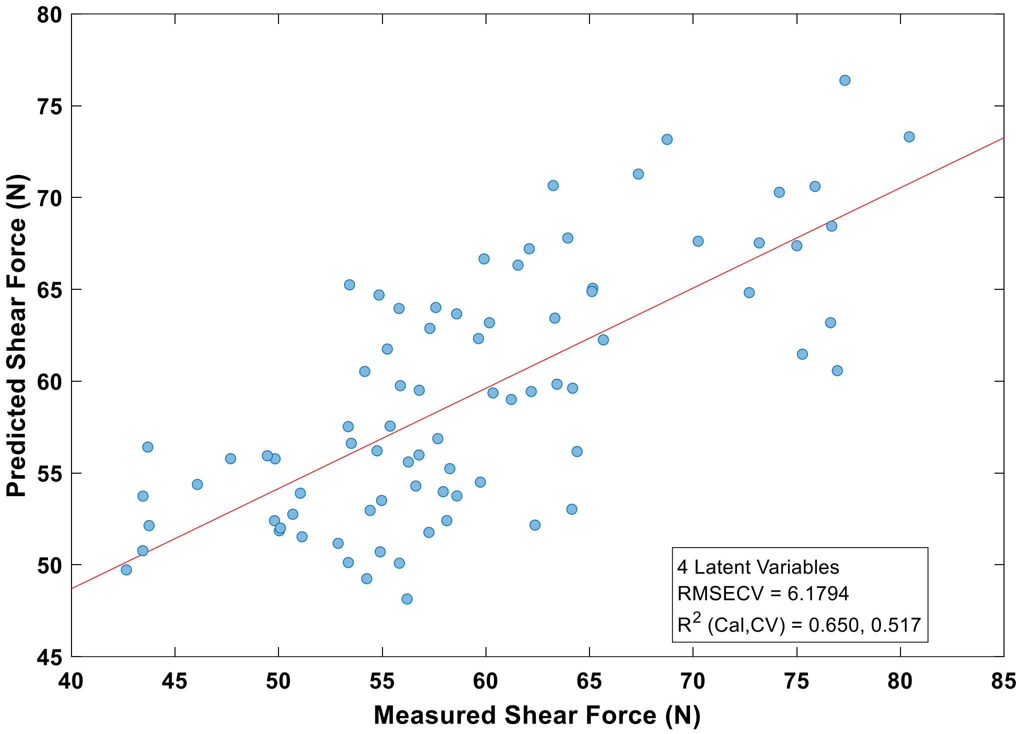

Figure 6. Shear force prediction using hide's spectra of location C ring 5. The RMSECV value is 6.2 and the R^2_{CV} = 0.52.

4.4 Prediction of soluble collagen content

4.4.1 Prediction of soluble collagen content from scans of eye round muscle

Table 9 summarises the results of models built using measurements obtained from rings 1 – 5 from eye round samples at 2, 14 and 28 days pm. In Table 7 It is observed that at all 3 ageing periods there is potential to predict soluble collagen from eye round spectra. The best performance is obtained when 2 day pm samples are used with the best performance from ring 5 measurements (R^2_{CV}=0.73, RMSECV = 0.44).

Figure 9 shows the prediction of soluble collagen of the eye round using ring 5 at 2 days post mortem illustrating the potential for predicting soluble collagen content using Vis-NIR measurements with appropriate choice of source-detector distance.

Table 9. Performance of models to predict soluble collagen using eye round spectra at 2, 14 and 28 days pm.

Ageing period	Ring	RMSECV	LV	R^2_{CV}
2 days	1	0.55	5	0.59
	2	0.81	5	0.70
	3	0.54	6	0.60
	4	0.44	7	0.73
	5	0.44	6	0.73
14 days	1	0.63	4	0.45
	2	0.62	3	0.47
	3	0.65	3	0.42
	4	0.63	4	0.46
	5	0.60	6	0.51
28 days	1	0.57	8	0.56
	2	0.58	7	0.54
	3	0.51	8	0.64
	4	0.57	7	0.56
	5	0.59	6	0.53

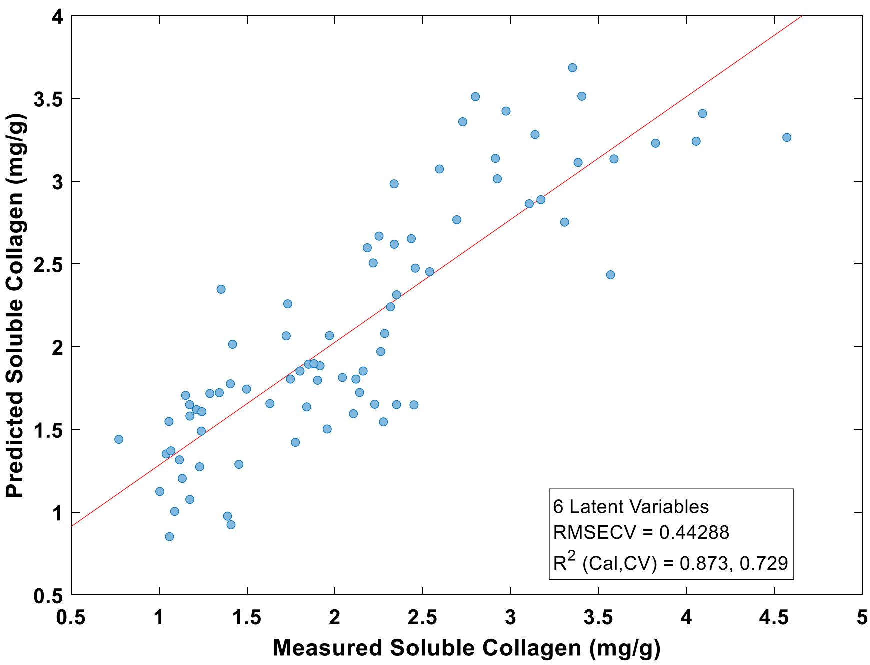

Figure 7. Prediction of soluble collagen using eye round muscle spectra from ring 5 at 2 days pm (RMSECV = 0.44, R^2_{CV} = 0.73).

4.4.2 Prediction of soluble collagen content from scans of striploin muscle

Table 10 summarises the results of models built using measurements obtained from rings 1 – 5 from striploin muscle samples at 2, 14 and 28 days pm. Only ring 5 indicated the ability to predict soluble collagen (R^2_{CV} = 0.44, RMSECV = 0.55). When measurements of other rings were used, the models were not able to predict the soluble collagen content. A similar observation was made regarding the prediction of shear force using striploin muscle spectra in section 4.3.2. It is not clear why a much better model can be built using measurements from ring 5. Given the result is very different from those obtained from the other rings, it has to be regarded as an anomaly and not taken to indicate predictive ability until further testing with additional samples.

Table 10. Performance of models to predict soluble collagen using striploin spectra collected at 2, 14 and 28 days post mortem.

Ageing period	Ring	RMSECV	LV	R^2_{CV}
2 days	1	0.71	2	0.08
	2	0.71	3	0.09
	3	0.73	5	0.09
	4	0.70	3	0.12
	5	0.55	5	0.44
14 days	1	0.72	1	0.05
	2	0.72	1	0.04
	3	0.72	1	0.05
	4	0.73	1	0.03
	5	0.73	1	0.02
28 days	1	0.70	3	0.10
	2	0.70	3	0.09
	3	0.70	3	0.10
	4	0.70	3	0.09
	5	0.71	3	0.08

It is no surprise that eye round spectra data indicated greater predictability of soluble collagen and shear force when compared to striploin spectra data. The eye round is a locomotive muscle and therefore requires a greater connective tissue content to support the movement of the leg.

Given the ability to predict age and shear force values from spectra collected on the eye round, it is hypothesised that the prediction of shear force values is an indirect measurement of the prediction of collagen content of the muscle. The ability of eye round scans to predict soluble collagen content further strengthens this hypothesis. Subsequently, as with the models to predict age, it is plausible that the accuracy of these models will be increased by including animals from a greater range of ages thereby improving the model calibration.

4.5 Prediction of marbling scores

Table 11 summarises the performance of models built using striploin muscle spectra at 2, 14 and 28 days pm. Only models based on samples at 2 days pm showed the potential for predicting the marbling scores with the best model performance arising from using ring 1 (R^2_{CV} = 0.49, RMSECV = 85). Rings 3 and 5 also showed some predictive ability. Figure 8 illustrates the correlation between the predicted and actual values of marbling scores.

Table 11. Performance of models to predict MSA Marbling scores using spectra of striploin muscle at 2, 14 and 28 days pm.

Ageing period	**Ring**	**RMSECV**	**LV**	**R^2_{CV}**
2 days	1	85.3	5	0.49
	2	96.2	4	0.35
	3	90.2	7	0.44
	4	98.6	3	0.31
	5	91.7	6	0.42
14 days	1	111.9	4	0.14
	2	104.5	5	0.23
	3	109.3	5	0.18
	4	107.5	5	0.20
	5	109.3	6	0.19
28 days	1	107.0	6	0.23
	2	106.4	7	0.23
	3	110.9	6	0.17
	4	109.8	3	0.18
	5	111.4	2	0.12

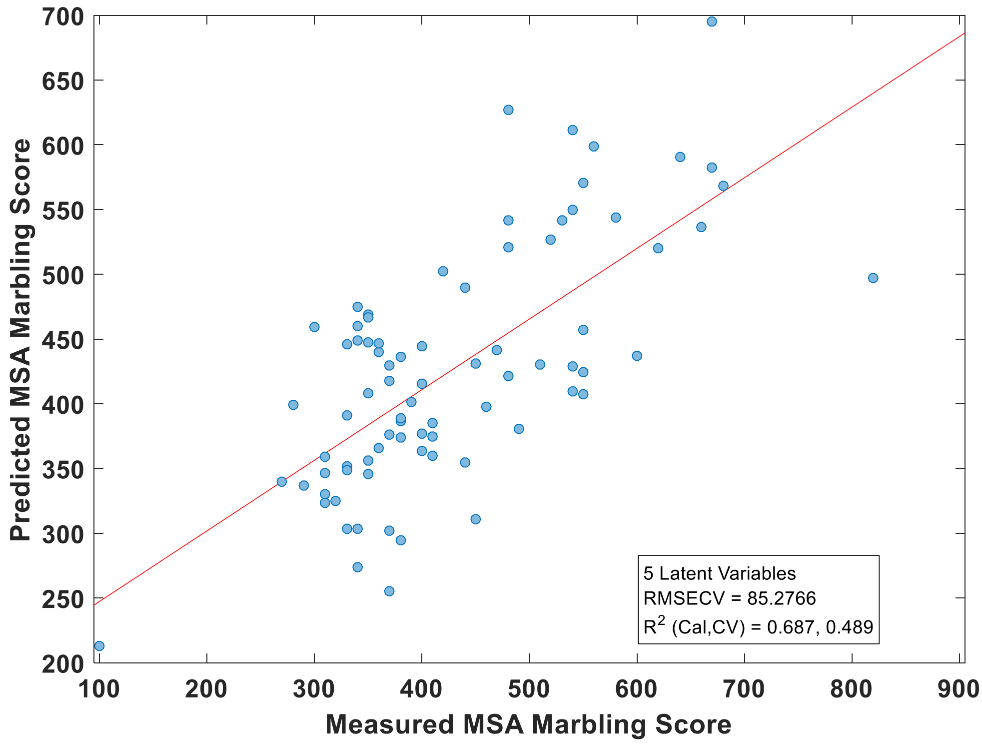

Figure 8. Prediction of MSA marbling scores of striploin spectra on ring 1 at 2 days post mortem (RMSECV = 85.3 and R^2_{CV} = 0.49).

5 Conclusions/recommendations

Overall, data collected and analysed to date indicates that the chronological age is best predicted using spectra collected on the neck region by ring 5. There was also potential to predict age using spectra from other rings and other locations such as location C (rib region) ring 5 and location A (neck) ring 1 also yielded low predictive errors and moderate accuracies. However, it must be acknowledged that this data set is limited by the large proportion of 2-3 year old cattle represented in the age categories which reduces the accuracy and increases the error associated with the models to predict chronological age. Thus, it is important that further cattle younger than 2 years and older than 3 years (up to about 12 years) are sampled to improve calibration models before further steps to industry adoption are taken.

Analysis of spectra data of muscles indicates that there is potential to predict animal chronological age by scanning the eye round muscle at 2 days pm. However, the ability to predict chronological age by scanning the muscles was decreased and the error associated with the prediction increased when compared to the ability to predict animal age by scanning the hides.

The prediction of physiological age through calibration models built using scans of hides to predict ossification scores was considered. Analysis suggests that the predictive ability of the scans is limited but this limitation is probably due to the lack of sensitivity of ossification scores for cattle over the age of 5-6 years. Similar results were obtained when muscle scans at 2 days post mortem were used with eye round muscle scans performing slightly better than the strip loin muscle scans.

It was found that shear force could be predicted from scans of muscle tissue with stronger evidence of this when eye round muscle scans were used. To obtain a more robust estimation of model error, again more samples spanning an age range from less than 1 year and over 5 years (up to around 12 years) will be required.

Models built to predict shear force from hide scans indicated some predictive ability particularly when scans from location C (rib region) collected by ring 5 are used.

As in the case of shear force prediction, scans of eye round muscles indicated the ability to predict soluble collagen content while striploin muscle scans did not indicate any ability to predict soluble collagen content except for the anamolous result from ring 5 scans which appeared to indicate the ability to predict soluble collagen. The prediction of the shear force values appears to be an indirect prediction of soluble collagen, which may explain the differences in prediction of shear force between the striploin and eye round muscles as connective tissue plays a major role in determining meat toughness in the eye round when compared to the striploin muscle.

Furthermore, analysis also indicated that striploin muscle scans have the potential to predict MSA marble scores when scans of samples are taken at 2 days pm.

While the results discussed in this report were based on models which incorporated wavelength selection by genetic algorithm, analyses were also carried out by building models without wavelength selection. In all cases, the models arising from application of wavelength selection led to lower root mean square error of cross validation. Given that the age distribution is skewed towards the age of 2-3 years and a relatively lower number of samples across other ages, there is potential for model overfitting to occur when applying GA especially since internal cross validation is used. This could lead to more optimistic estimates of predictive ability than warranted. Therefore, it is essential to collect additional sets of data with a larger range of animal ages to include more cattle below 2 years of age and more cattle older than 5 years of age thus providing an approximately uniform distribution of ages. Such a dataset will allow a more robust calibration model to be built. Additionally, a sufficient number of samples that span the desired age range can be set aside to be used as an external validation (i.e. an independent test) set so that a reliable estimate of predictive ability of the models can be obtained.

Analysis indicates that, in most cases, spectra from ring 5 lead to models that are better compared to measurements from other rings. This raises the possibility that, in the future it may be possible to simplify the probe design and thus reduce the cost of the device and possibly the measurement time. Also, based on the analyses carried out, the number of locations from which data will be collected in the future can be restricted to locations A (neck) and C (rib region).

6 Key messages

- The potential for predicting chronological age of cattle has been demonstrated when PLS calibration models combined with wavelength selection is used.
- Similarly, the potential to predict meat quality traits (shear force, soluble collagen content and marbling scores) has been demonstrated.
- It was difficult to obtain samples from cattle with known birth dates spanning a broad age range (0 – 12 years) which is required to build robust calibration models.
- The use of wavelength selection with internal calibration could lead to more optimistic estimate of model error. Therefore a sufficient number of samples need to be collected that span the required age range and allows for adequate number of samples spanning the age range of calibration to be set aside as an external validation set.

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