final report

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NMR Measurement of Intra-Muscular Fat

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Executive summary

The time-domain NMR system "Oscar 2" was shipped to the University of New England, Armidale, NMR where it was used to measure lamb loin samples for fat content. The practical work was completed June 5-7, 2019. Data was collected at 12.3 MHz on lamb longissimus dorsi samples from 239 lambs as part of the ALMTech programme. The corresponding intramuscular fat (IMF) data were also acquired (by an laboratory infra-red method) and used to build predictive models that had a predictive correlation of 0.84 (r2= 0.71) over a range of 3-8% IMF with a predictive error of 0.5%.

This error was similar to the uncertainty in the NMR fit parameter and primary indicatory p_{2f}. The prediction models are only dependent on the NMR parameter p2f, which is the amplitude of the short exponential decay. The uncertainty in IMF prediction is most likely due to an oscillation at the beginning of the CPMG decay, which is most likely caused by an instrument artefact that can be fixed. This would potentially remove instrument error as a significant source of variance. The performance of the predictive model reported here performs similar to, if not better than the models reported from a previous AgResearch project. However, different models are required, possibly because of the species involved.

The methods used were based on prior work conducted for MLA – P.PSH.0878. For context on the NMR measurement and the equipment refer to Appendix 1.

Table 1. Summary of model correlations and predictive errors

Method	**Model**		**Cross-validation**	
	r (r^2)	RMSEP	r	RMSEP
NMR signal normalized to 1				
p_{2f} linear model	0.84 (0.70)	0.51	0.81	0.51
p_{2f}, p_{21}, p_{22} linear model	0.84 (0.70)	0.51	0.83	0.53
PLS model using CPMG data*			0.83	0.54
NMR signal normalized by the sample weight				
p_{2f} linear model	0.77 (0.59)	0.61		
p_{2f}, p_{21}, p_{22} linear model	0.84 (0.70)	0.52	0.84	0.53
PLS model using CPMG data*			0.83	0.54

* 7 component

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1 Background

Accurate measurement of intra-muscular fat (IMF) under commercial abattoir conditions is an import R&D goal for both the beef and sheepmeat industries. The beef industry currently subjectively assesses marbling (a proxy for IMF) by AUS-MEAT accredited graders. The lamb industry currently has no individual carcase, or cuts-based, quality grading procedures. Research conducted by the Sheep Cooperative Research Centre has developed a cuts-based MSA grading model to enable the lamb and sheepmeat industry to adopt individual carcase cuts-based grading, as is done by the beef industry. However, this new MSA quality grading model requires IMF to be implemented. MLA and industry collaborators are investigating a number of technologies to measure IMF. Success will enable the cuts-based MSA grading model for lamb and sheepmeat; and provide a new objective input into beef MSA grading

2 Project objectives

The project was conducted as small pilot to test the potential of NMR to measure IMF in lamb. This created a dataset to complement and compare findings in beef with the objective of informing further investment in this technology.

3 Methodology

NMR Data was collected on the Oscar 2.0 NMR system running at 12.3 MHz with the magnet temperature set to a few degrees above room temperature of 20^{o} C and controlled by a heat pump. A lamb *longissimus dorsi* sample of about 12-18 g was cut from the portion of meat set aside for intramuscular fat analysis. Samples were measured in batches and stored at 1^{o} C when not being measured. Sample temperature was not closely controlled or monitored. Samples were measured 3-5 days after slaughter and between hours and days of being butchered. Of the 241 lambs slaughtered, 239 samples were used for creating the model.

During the analysis, CPMG maximum amplitudes were either normalized to unity or by unit sample weight. CPMG data was fitted to three exponentials using the equation:

$$I = \sum^{i} p_{2i}\, e^{-\frac{t}{T_{2i}}}$$

where *I* is the signal intensity, p_{2i} the exponential amplitude, T_{2i}, the relaxation time and subscript *i* designates the population components *f*, *1*, or *2*. T_{2f} was fixed at 10 ms, while all other amplitudes and time constants were minimized through fitting. IMF prediction models were created either from linear models of the exponential fit results or from PLS analysis of the CPMG data.

The probe was repaired partway through measuring the samples. Analysis of the data does not suggest that this adversely affected the measurements.

4 Results

4.1 Correlation of IMF with NMR parameters

IMF is highly correlated to the different exponential amplitudes, with the highest of 0.84 to p_{2f}. The other correlations are slightly lower than their respective correlations with p_{2f} and therefore, suggest that they are derived indirectly from their correlation with p_{2f}.

Table 2. Correlations of NMR parameters to IMF

Parameter	Correlation
p_{2f}	0.84
p_{21}	-0.51
T_{21}	-0.07
p_{22}	0.32
T_{22}	0.17

4.2 Linear fits

Due to the high correlation of IMF to p_{2f}, this linear relationship was fitted to a first-order polynomial. This fit is shown below in Figure 1 and has a correlation of 0.84 and a root-mean-squared error (RMSE) of 0.52 %. The fit model and statistics are shown in detail below Figure 1.

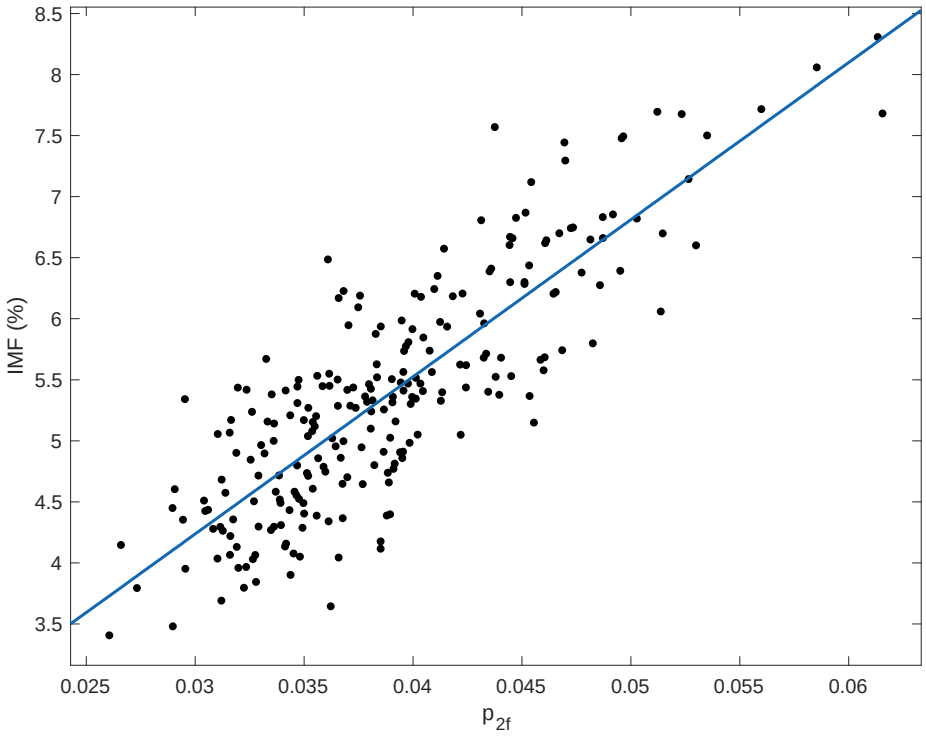

Fig. 1: The first-order polynomial fit of IMF as a function of p2f from fitting NMR data to three exponentials.

Linear model Poly1:
f(x) = p1*x + p2
Coefficients (with 95% confidence bounds):
p1 = 128.7 (118, 139.4)
p2 = 0.3747 (-0.0464, 0.7958)

Goodness of fit:
SSE: 65.21
R-square: 0.7036
Adjusted R-square: 0.7023
RMSE: 0.5246

4.3 Linear Predictive models

A linear prediction model was created using only the p_{2f} fit parameter, the addition of more parameters decreases the predictive power of the model. The model was cross-validated by fitting 50 randomly chosen samples and predicting the remaining 189 over 2500 iterations. The results were a predictive correlation of r=0.84 and root-mean-squared error of prediction (RMSEP) of 0.53 %. The mean prediction values are plotted against the measured values below in Figure 2. The inclusion of p_{2f}, p_{21}, and p_{22} results in a marginal improvement in the RMSEP to 0.51% and no change in predictive correlation.

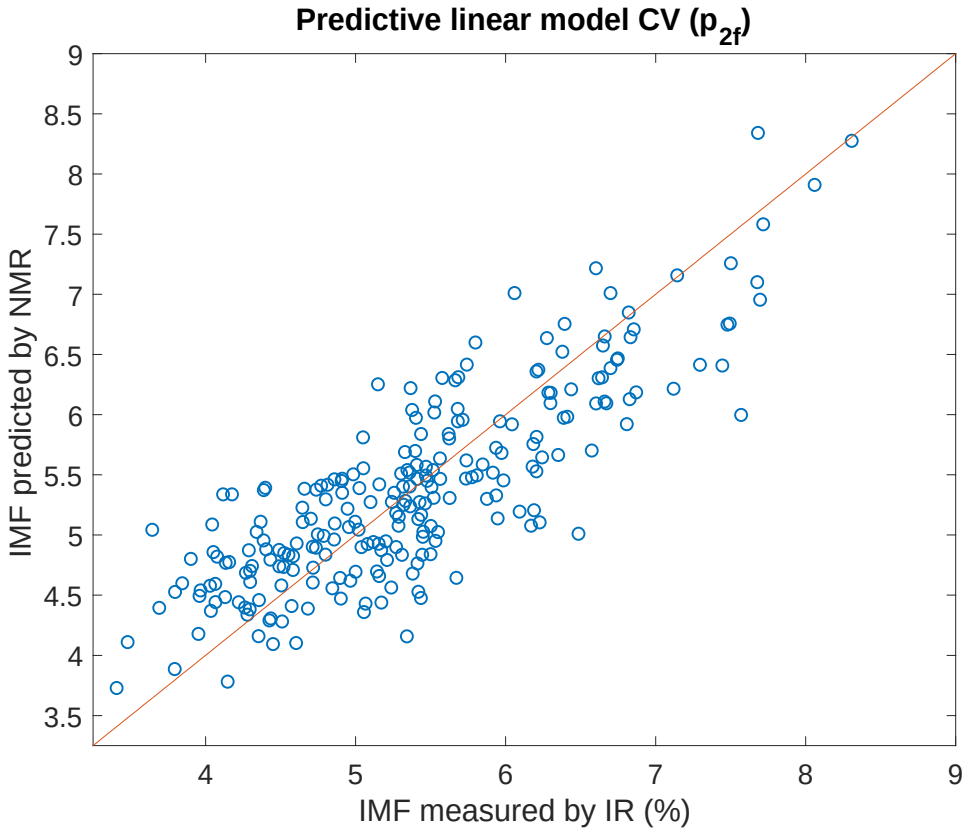

Fig. 2: Predicted values from cross-validation of the linear model created from p_{2f} and IMF

4.4 PLS model

A PLS model was developed to predict the IMF. The optimal model required 7 components and accounted for 75% of the variance in the data. Cross-validation required 189 samples for creating the model while the remaining 50 were used for prediction. The predictive correlation and error in IMF were determined to be r = 0.83 (r^2 = 0.69) and RMSEP = 0.54% from 1000 iterations of model generation and prediction. The mean prediction values are plotted against the measured values below in Figure 3.

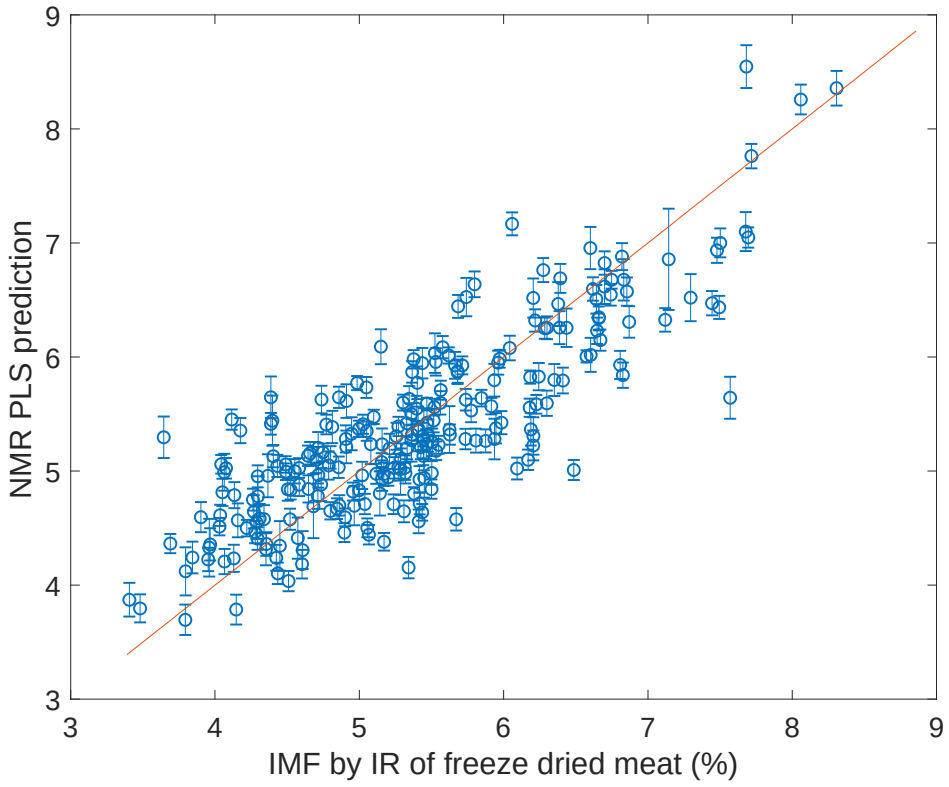

Fig. 3: Predicted values from a PLS model created from CPMG data normalized to unit maximum amplitude in the CPMG and IMF.

4.5 Normalizing the CPMG signal to sample weight

The CPMG decays were normalized by the sample weight and linear and PLS were tested. Due to the high correlation of IMF to p_{2f}, this linear relationship was fitted to a first-order polynomial. This fit is shown below in Figure 4 and has a correlation of 0.77 and an RMSE of 0.62 %. The fit model and statistics are shown in detail below Figure 4. This error is greater compared to the NMR data that is normalized to unit amplitude. When p_{21} and p_{22} are added to the model a similar performance to the unit amplitude NMR data is achieved. This suggests that a relative amplitude is required for fat prediction.

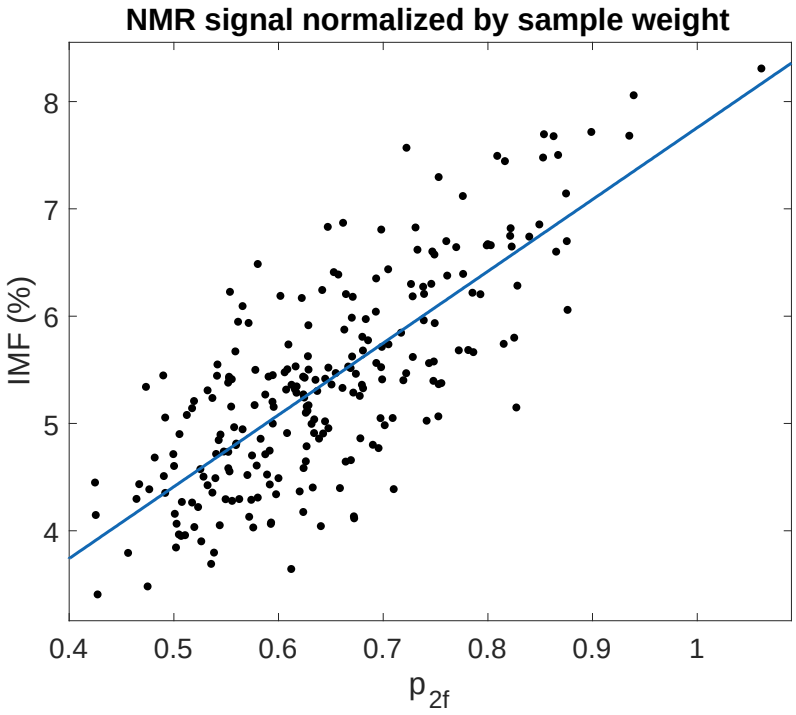

Fig. 4: The first-order polynomial fit of IMF as a function of p_{2f}, where the NMR data were normalized to sample weight.

Linear model Poly1:
f(x) = p1*x + p2
Coefficients (with 95% confidence bounds):
p1 = 6.69 (5.974, 7.406)
p2 = 1.066 (0.5973, 1.534)

Goodness of fit:
SSE: 90.53
R-square: 0.5885
Adjusted R-square: 0.5868
RMSE: 0.618

4.6 Linear predictive models

A linear prediction model was created using p_{2f}, p_{21}, and p_{22} fit parameters. The model was cross-validated by fitting 50 randomly chosen samples and predicting the remaining 189 IMF values over 2500 iterations. The results were a predictive correlation of r=0.84 and RMSEP= 0.52%. The mean prediction values are plotted against the measured values below in Figure 5. (The model coefficients were 5.92, 7.43, -0.37, and -0.29 for offset, p_{2f}, p_{21}, and p_{22} respectively.) A PLS model (not shown) did not improve the predictive power (r = 0.83, RMSEP = 0.54%).

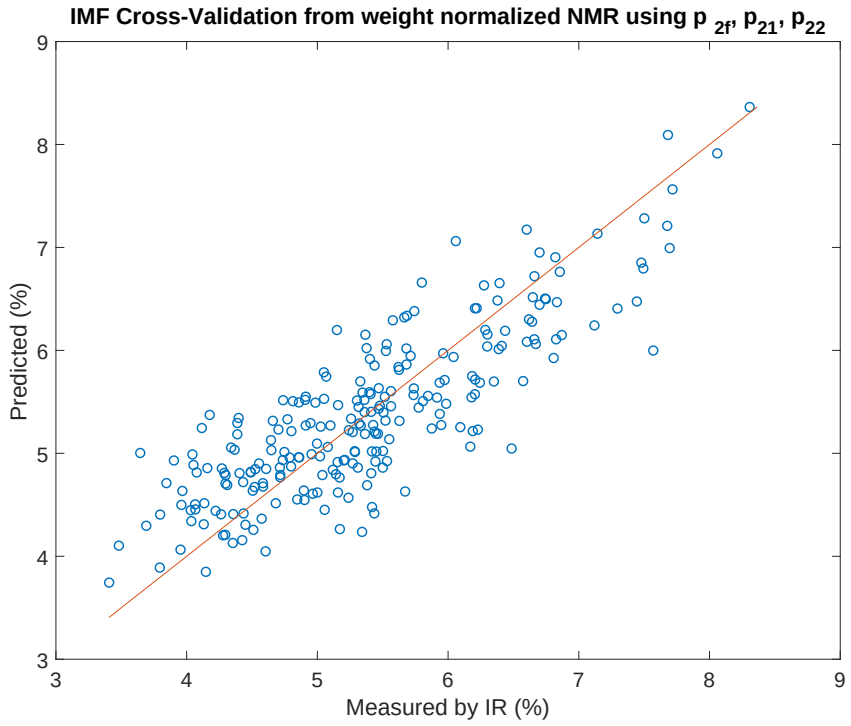

Fig. 5: Predicted values from a linear model created from p_{2f}, p_{21}, and p_{22} and IMF where the NMR data was normalized by the sample weight

5 Discussion

The models produced here generally result in a correlation of 0.84 and RMSEP of 0.5%. This is achieved with a simple linear model using p_{2f} obtained from fitting CPMG data normalized to unit maximum intensity. Models that included more fit parameters did not improve the predictive correlation. The use of the data reduction method, partial least squares, (PLS), did not improve the predictive power either. This suggests that all the information is included in the parameter p_{2f}. This agrees with previous studies we have done (AgResearch and MLA[1]). It also agrees with the chemico-physical model that fat will have a faster and resolvable T_2 population from the water within meat. The discussion considered the origin of the dominant error and how this study compares to the AgResearch dataset.

5.1 Analysis of error

An RMSEP of 0.5% was observed over a range of 3-9% IMF, which is roughly an 8% relative error in the predictive models. These models are based on p_{2f} that has a relative uncertainty of ~9% in the three exponential fit model. Therefore, we believe that the predictive error is dominated by the uncertainty in p_{2f}. It is also reasonable to attribute this uncertainty to an oscillation that occurs at the beginning of CPMG data that has an amplitude of about 5-10% of the signal in the first few milliseconds of data, as shown in Figure 6. If this oscillation were on the scale of the signal to noise, the relative error in p_{2f} would be about 0.03 %. This oscillation is likely due to poor RF amplifier performance creating out of phase signal that takes time to lose coherence. Whatever the cause, it is safe to say that it can be resolved because very similar oscillations have been observed in other systems and subsequently fixed. It is very likely that there will be a residual oscillation related to field homogeneity, but this will be much smaller than what we are currently observing.

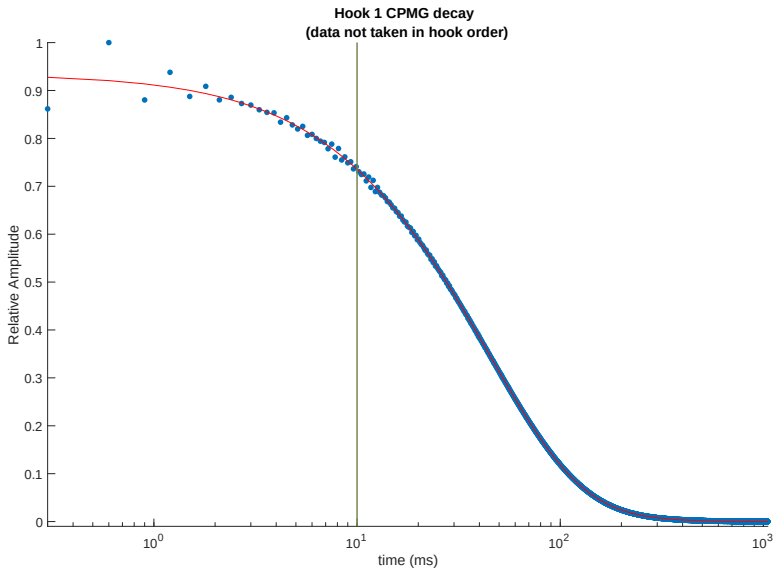

Fig. 6: The CPMG decay collected for the sample that came from Hook 1.

[1] P PSH 0878 - Evaluation of eating quality attributes measured by TD-NMR.

The predictive error was also analysed by sample number to see if any trends were recognizable. Figure 7 shows the magnitude of the predictive error and a moving mean over 5 samples. The samples were not measured in the order of hook number, however, clumps of samples with similar hook numbers were usually measured at similar times. For example, 29 and 72 were mostly measured on the last day after all the probe issues were sorted. These samples were also freshly butchered as we were measuring them. Possible sources of error are instrument error or loss of water between when the sample was prepared and measured.

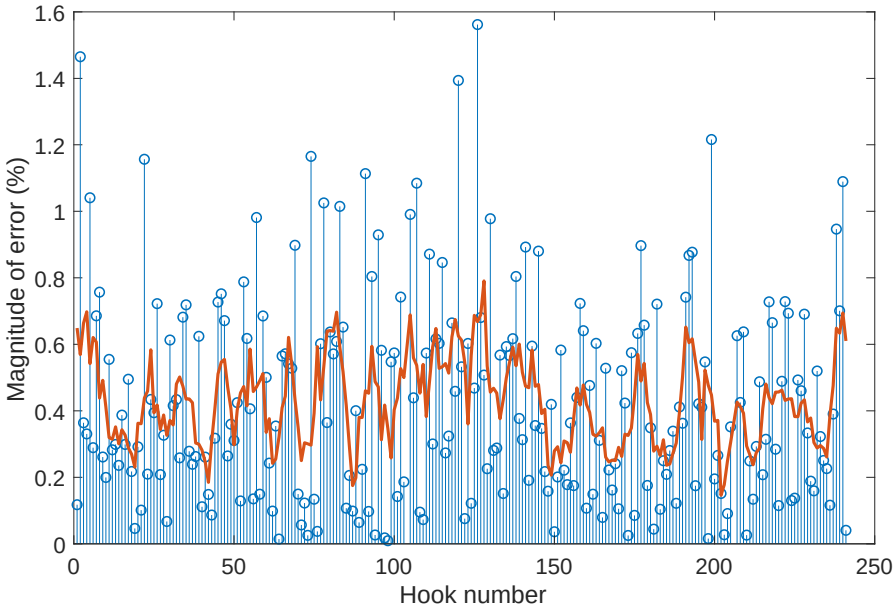

Fig. 7: The uncertainty in predicted IMF by sample number with a moving mean of 5 samples plotted on top.

5.2 Comparison of models to AgResearch trial

As a reference, an AgResearch project (Wagyu and bull beef) had a correlation of 0.96 (r^2 = 0.92) over a much larger range (1-19%), but the RMSEP was 1%. The Wagyu on its own had a correlation of r=0.88 (r^2 = 0.78), but only an RMSE of 1.8%. When predicting numerical values, it is better to look at the predictive error and determine if that is suitable for the application. One reason that may have resulted in a lower error for the ALMTech data is that the fat content was measured on samples that were made up of mostly (~75%) the same sample measured by NMR. For the AgResearch project, samples that were different but considered equivalent were used to measure IMF.

Another aspect of the comparison is that different models are required for lamb and Wagyu and possibly still another for bull (or lean) beef (Figure 8). This leads to the question of how general are the IMF models. Will a new model be needed for each species, breed, or sample set? However with such a simple model, it is easy to create a new model with a limited data set.

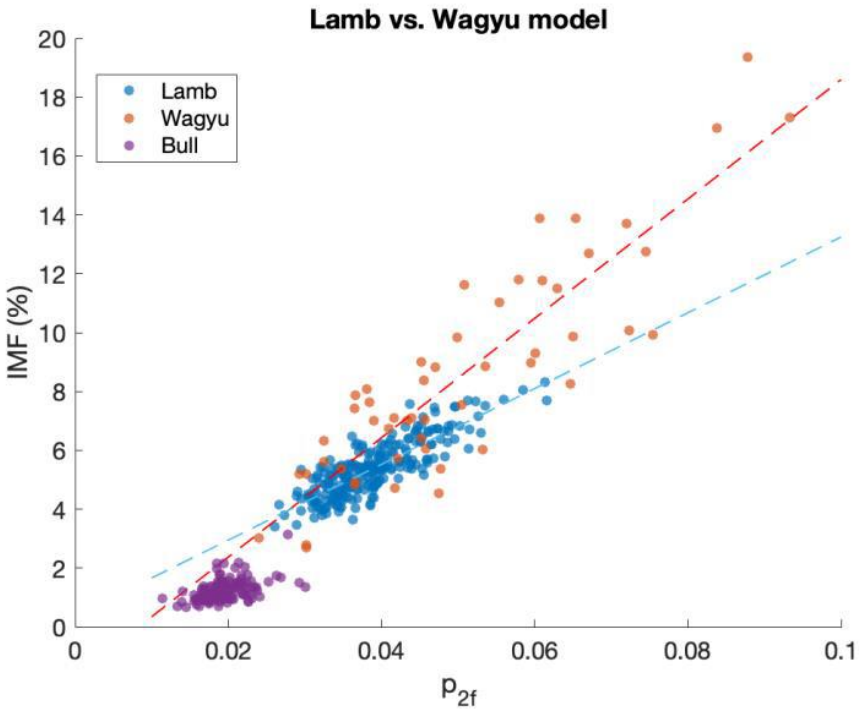

Fig. 8: Different correlation models may be needed for different meat or breed, as suggested by comparison of data from recent AgResearch and MLA trials on beef, and the current trial on lamb.

6 Conclusions/recommendations

The data reported from this analysis of lamb loins from the MLA Resource Flock builds on that from MLA project P.PSH.0878 and beef results from an AgResearch (NZ) trial. These data support the potential for time-domain nuclear magnetic resonance to measure IMF in both lamb and beef.

The current NMR method requires a 15g sample to be inserted into an enclosed magnetic field zone for analysis in a laboratory environment. It is recommended that any further investment should focus on development and pre-commercial testing of a prototype 'single-sided' NMR configuration to assess the accuracy and precision of IMF measurement from a configuration that could potentially be applied to the loin of an un-cut lamb or beef carcase.

7 Appendix

7.1 What is NMR?

Nuclear magnetic resonance spectroscopy (NMR)[2] was first developed in 1946 by research groups at Stanford and M.I.T., building on radar technology developed in WW2. Over the next 50 years NMR developed into the premier organic spectroscopy available to chemists to determine the detailed chemical structure of the chemicals they were synthesizing.

Many atomic nuclei possess permanent magnetic moments (or "spin", like a gyroscope) and, when placed into an external magnetic field, tend to align themselves along the field. The most often-used nuclei in NMR are hydrogen-1 and carbon-13, although certain isotopes of many other elements nuclei can also be observed. The magnetic moments of all nuclei present in a sample sum up to a macroscopic vector quantity called nuclear magnetization. In equilibrium, nuclear magnetization is aligned along the magnetic field and, being tiny and static, is almost impossible to detect against the main field background.

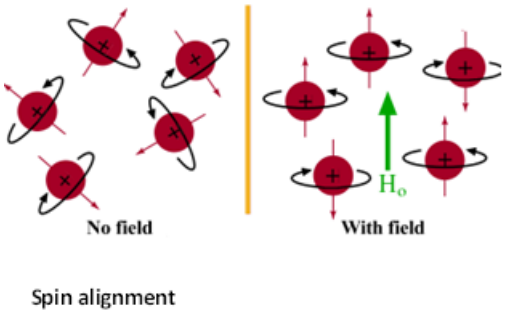

Fig. 1: Alignment of nuclear spins in an external magnetic field

In an NMR experiment the magnetic alignment is perturbed using a radio-frequency pulse, where frequency is proportional to the external field strength for a given nuclei. In returning to equilibrium the nuclei re-emit radio frequency energy which can then be detected by a nearby receiver coil.

[2] This description is adapted from http://www.ebyte.it/library/educards/nmr/OnePageMrPrimer.html and other on-line sources.

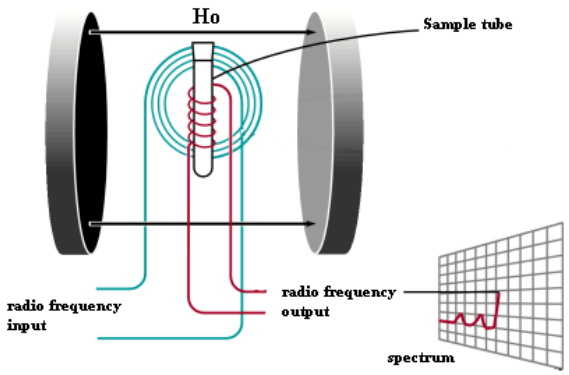

Fig 2: Schematic of an NMR experiment in which a radio frequency input (blue coil) perturbs the nuclear magnetisation produced by the external field H_0, the relaxation of which can be detected (red coil).

The time required for the nuclei to return to equilibrium after excitation is the "relaxation time" and relaxometry NMR (or time-domain NMR) involves the measurement of these times, and is typically done at relatively low applied magnetic field strengths. T1 relaxation, also called spin-lattice or longitudinal relaxation, relates to energy dissipated to the surrounding molecular framework; T2 relaxation, or spin-spin or transverse relaxation, relates to energy dissipated to neighbouring nuclei. Nuclei in different environments have different T1 and T2 relaxation times, hence the NMR relaxometry method has the ability to discern different populations (e.g., p_{2f}) of the given nucleus (^{1}H in water and fat relevant examples in the present case).

This response is also exploited in magnetic resonance imaging ("MRI") and also in high-field spectroscopy where chemical structures can be elucidated by transformation of the time-domain signal into the frequency domain, in the latter case the small differences between the magnetic fields experienced by individual atomic nuclei due to their chemical environment, including those produced by the presence of electrons and those due to interactions with close-by nuclides of the same or different kind and which may be mediated by chemical bonds, are detected. Being very small, these field variations are measured *at most* in parts per million (ppm) with respect to the external field, down to tiny fractions of a ppm.

The NMR system employed in the present research consisted of a temperature stabilised permanent magnet array of Halbach configuration giving a uniform field transverse to sample entry direction (called "Oscar 2.0" as seen in Figure 3 below). The sample container consisted of a common plastic bottle with screw cap attached to a suspension rod (Figure 3). The sample space was kept chilled via the use of a thermoelectric cooler mounted above the magnet. A Kea spectrometer developed by Magritek (Wellington, NZ) and personal computer completed the NMR instrument.

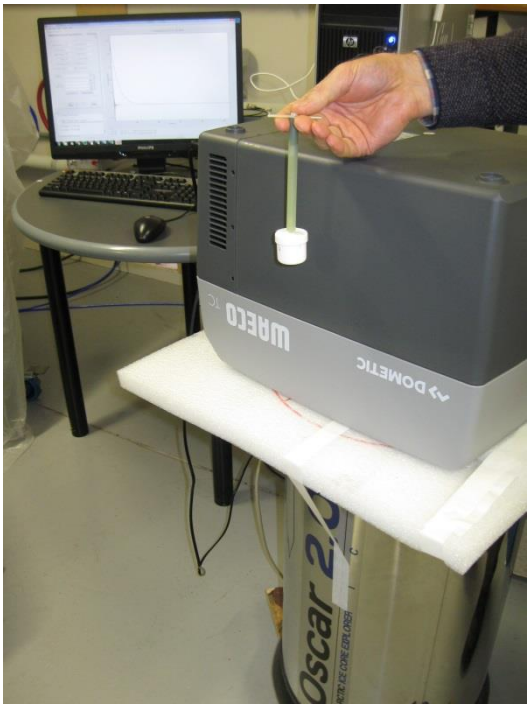

Fig. 3: the NMR system with sample holder in front of thermoelectric chiller sitting on the magnet ("Oscar 2.0").