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Preliminary study of a Magritek low field nuclear magnetic resonance device to measure meat attributes

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Acknowledgement

We wish to acknowledge the support of Dr Andrew Coy and Dr Craig Eccles of Magritek who developed the open-topped low field NMR device called the Kea control system and also the Terranova system and set it up for use in this work. The magnet configuration of the Magritek device allows placement of samples on top of the sensing unit, which has many advantages over existing LF-NMR devices. Although their services were provided under another contract, without their help, we could not have undertaken these experiments. We believe the synergy has been beneficial as we have freely been able to discuss the various options and potential improvements to the equipment. This cooperation is essential in developing new equipment and concepts.

Executive summary

- Nuclear magnetic resonance (NMR) technology has been used to study red meat quality primarily in research applications. Development of a one sided low field (LF) NMR instrument by the New Zealand company Magritek offers the possibility that LF-NMR technology could be used in a commercial “on line” application.
- • This report details an experiment conducted collaboratively by AgResearch MIRINZ and Murdoch University at Ruakura Research Centre in Hamilton, New Zealand. The aim of this experiment was to investigate the suitability of the Magritek LF-NMR system for measuring meat quality as a precursor to development of a commercial application.
- • An experiment was designed with 2 treatments, electrical stimulation and no electrical stimulation to create variation in meat quality attributes between samples and over time from slaughter. The loins of 70 lambs slaughtered in 7 kill groups of 10 were held at 15°C to accelerate ageing. Data for a range of meat quality attributes including pH, glycogen concentration, water holding capacity and tenderness were compared to LF-NMR readings.
- • As expected there were significant trends between samples and over time for all meat quality attributes. This variation confirmed that the experimental design was suitable for testing the LF-NMR instrument.
- • A weak trend was detected in time from slaughter for T2 relaxation times but this trend was found only with mean data and not with individual sample data. Correlations between T2 relaxation times and meat quality data were poor.
- • The signal to noise ratio for this instrument was found to be low. The conclusion was made that this characteristic was likely to be responsible for the difficulty in detecting changes in T2 data with time and correlating against meat quality data with T2 data.
- • Evidence suggested that temperature control of the magnet was at least partly responsible for this result and the strength of the magnetic field may also be too low. Magritek is in agreement over these issues and they have undertaken to make changes to the instrument to deal with these issues.
- • The conclusion was made that further refinement of the Magritek one sided low field instrument is required before further work should be undertaken for an “on line” meat quality measurement application.
- • Improvements by Magritek are currently underway as a result of this experiment and further meat quality work using the refined LF-NMR devices is recommended.
- The Magritek Terranova unit was also tested in this experiment but was shown to be inappropriate for the measurement of meat quality traits. This unit was not capable of detecting short relaxation times such as those in meat. The unit may be applicable to measure attributes in more uniform consistency such as blood or oil.

Contents

1 Introduction

On-line measurement of meat quality is expected to be a useful tool that will allow product description and facilitate process control and management. Nuclear magnetic resonance (NMR) is one measurement technology that may potentially be suitable for development into commercial applications for red meat processing but this requires further investigation.

A NZ based manufacturer (Magritek) has developed an open-topped low-field (LF) NMR prototype device that allows the placement of samples on top of the sensing unit (see Figure 1). This enables the measurement of larger samples than is possible with bench top LF-NMR units and eliminates the need to cut a sample from the product. Although available commercially, it is unclear as to whether the Magritek instruments will be capable of measuring meat quality attributes. In addition, it is unclear whether parameters related to diffusion or mobility are the most appropriate to assess meat quality attributes using LF-NMR.

Figure 1. Magritek Kea LF-NMR device (control system on left, magnet on right)

Water within meat is contained in several different compartments including the extra cellular and intracellular spaces as well as being tightly bound to protein. The relative location of water depends on factors such as pH, sarcomere shortening and protein characteristics (Guignot *et al*., 1993). Hence measurement of water characteristics using NMR instruments might be a way of predicting meat quality characteristics such as pH, glycogen content, tenderness and water holding capacity. There is evidence in the literature that water movement between cellular compartments associated with attainment of rigor and cooking can be measured with LF-NMR benchtop instruments. Bertram *et al.* (2001) discovered a correlation with a high R^2 between LF-NMR and water holding capacity. She also studied several other meat properties including pre- and post rigor changes. Elisabeth Micklander, an author of this report, has also used benchtop LF-NMR instruments to study changes in meat during cooking (Micklander *et al.,* 2002) and in pre-rigor meat (Micklander *et al.,* 2005).

The aim of the experiment described in this report was to examine the various NMR parameters and to compare the LF-NMR readings against meat quality attributes such as tenderness, water holding capacity, glycogen content and pH for the ultimate purpose of measuring meat quality using LF-NMR.

2 Materials and methods

2.1 Animals and treatments

A total of 70 lambs approximately 12 months old, in 7 kill groups of 10 lambs each, were used in this experiment. In four of the kill groups (1, 3, 5 and 6) the lambs were electrically stunned from head to back (3 s at 500V) and underwent electrical stimulation at 80 V peak, 14.28 pulses s^{-1} for 30 s, post dressing. In the remaining three kill groups (2, 4 and 7) the lambs were shot with a captive bolt and dressed without subsequent electrical stimulation (Table 1).

Table 1. Sample numbers and treatment for samples in kill groups 1 to 7.

Kill group	Sample Numbers	Electrical Stimulation?
1	1-10	Yes
2	11-20	No
3	21-30	Yes
4	31-40	No
5	41-50	Yes
6	51-60	Yes
7	61-70	No

2.2 Samples

The *m. longissimus lumborum* (LT) were removed from both sides of each carcass on entry to the chiller approximately 30 minutes after slaughter and taken to the AgResearch MIRINZ laboratory 800 m away.
The LT's from both sides were tightly wrapped in 4 layers of cling film, placed in waterproof polyethylene bags and then immersed in a water bath set at 15°C.

When the muscle temperature reached 15°C, about 3 hours post slaughter, they were removed from the water bath and placed in a 15°C room for the duration of the experiment.

When readings were made during the period that the samples were held in the water bath, the samples were removed one at a time for measurement and returned to the water as soon as the measurement was completed.

2.3 LF-NMR measurements

Each LF-NMR reading consisted of four Carr-Purcell-Meiboom-Gill (CPMG) experiments conducted consecutively at each sampling time using the parameters given in Table 2.
Table 2. Parameters of four CPMG experiments carried out consecutively at each LF-NMR measurement.

Experiment	Echo time	Number of Points	Number of scans
1	0.14 ms	512	32
2	0.30 ms	512	32
3	1.00 ms	256	32
4	5.00 ms	64	32

The B1 frequency was optimised for each sample to make adjustment for small changes in room and magnet temperature between samples. A frequency sweep was conducted prior to starting each LF-NMR reading. This sweep obtained the optimal B1 frequency for use on that sample at the beginning of the reading.

The Magritek Terranova unit was also to be evaluated in this study but initial testing indicated that the unit was inappropriate for use on meat. Further details of this are located in Appendix 3.

2.4 Sampling Procedures

The sampling times, procedures and loin sections used are detailed in Table 3.
The pH was recorded using a Mettler Toledo pH meter with combination electrode. The time point at which *rigor mortis* would occur was difficult to predict. To overcome this difficulty, it was defined as when the pH fell below 5.6 for normal pH muscles or when the pH ceased to fall for muscles with elevated ultimate pH values. Muscle temperatures were measured with Dallas iButtons accurate to ±1 °C. Spot measurements of temperature were also taken to ensure the meat reached the designated 15°C when chilling in water 10-15°C and to provide constant ageing conditions.

LF-NMR measurements were performed using a Magritek (Wellington, New Zealand) Kea one-sided magnet instrument (see Figure 1) with a resonance frequency of approximately 3 MHz. LF-NMR measurements were performed at 11 nominal time points (TP1-TP11) as described in Table 3.

The first quarter (starting at the rump end) of the left loin was cut off prior to LF-NMR readings to avoid the loin acting as an antenna leading the NMR signal away from the NMR coil. The first three LF-NMR readings were performed on this quarter of each left loin at specific time points and after each reading small samples (~2g) were taken from this quarter and immediately frozen in liquid nitrogen to be later analysed for glycogen content. The fourth LF-NMR reading, also on the first quarter of each left loin, was taken when the first loin in the kill group reached pH 5.8. After the fourth reading, the first quarter of each left loin was taken for tenderness and water holding capacity measurements. Thereafter one quarter of each left loin was used for each measurement until none was left. The sampling then took place on the right loin, also in quarters starting from the rump end. The total ageing duration for each kill group was approximately 60 hours at 15°C.

2.5 Measurements of glycogen content, water holding capacity and tenderness

At each time point where glycogen content, water holding capacity and tenderness was required the necessary samples were taken.

For glycogen concentration, each frozen sample was powdered while still frozen and a 1g sub-sample was then analysed using the perchloric extraction and colorimetric analysis method described by Krisman (1962).

For water holding capacity analysis three accurately weighed replicate samples of approximately 0.5 g were removed from the loin quarter. The samples were analysed for water holding capacity by the filter paper press method described by Van Oeckel *et al*. (1999). Water holding capacity measurements were not obtained over the pre-rigor period; however, the measurements taken at rigor would include any effects related to pre-rigor temperature conditions.

For tenderness analysis the loin quarter at each time point was immediately frozen. The samples were later cooked from the frozen state in a 100°C water bath until an internal temperature of 75°C was reached (measured by a thermocouple) and then immediately placed in ice-water slurry. Once cooled, 10 mm x 10 mm cross section samples (n=10 from each sample) were cut out and sheared with a MIRINZ Tenderometer.

Table 3. Overview of sampling times, loin number used and samples/readings taken.

Time point	Nominal time	Left/right side	Quarter used (1=rump end, 4=head end)	Samples/readings taken
1	~ 1h p.m.	Left	1	pH, LF-NMR, Glycogen
2	3 h p.m.	Left	1	pH, LF-NMR, Glycogen
3	6/9 h p.m. for ES/NES	Left	1	pH, LF-NMR, Glycogen
4	Taken when first loin in kill group reached pH 5.8	Left	1	pH, LF-NMR, Glycogen, Tenderness, Water holding capacity
5	1.5 h after time point 4	Left	2	pH, LF-NMR, Tenderness, Water holding capacity
6	3.0 h after time point 4	Left	3	pH, LF-NMR, Tenderness, Water holding capacity
7	4.5 h after time point 4	Left	4	pH, LF-NMR, Tenderness, Water holding capacity
8	6.0 h after time point 4	Right	1	pH, LF-NMR, Tenderness, Water holding capacity
9	12 h post rigor	Right	2	pH, LF-NMR, Tenderness, Water holding capacity
10	37 h post rigor	Right	3	pH, LF-NMR, Tenderness, Water holding capacity
11	61 h post rigor	Right	4	pH, LF-NMR, Tenderness, Water holding capacity

2.6 Data analysis and statistical procedures

Data analysis was performed on the raw relaxation curves and on parameters obtained from discrete exponential fitting of the curves, in particular the initial signal intensity (M2) and the relaxation time (T2) - see Appendices 1 & 2 for further information. Two approaches were used to analyse the data:

1. A chemometric approach using principal component analysis (PCA) and partial least squares (PLS). See Appendices 1 & 2 for information on PCA and PLS.
2. A statistical approach where a linear mixed model was used to fit a repeated measurements model to the relaxation time data (T2 and M2). The GenStat® (version 9.1 http. http://www.genstat.com) procedure REML was used in the model. The models can be described in a GenStat® formula where Time is the repeated measurements factor. Electrical stimulation is in the model as a factor with one of 2 levels (NES and ES). A power correlation model was used with equally spaced data points.

3 Results and discussion

3.1 Raw data: Meat attributes

The raw data collected for pH, glycogen concentration, water holding capacity and shear force are shown in Figures 2 to 5. These figures demonstrate that the experimental design was successful in creating a large range in meat quality attributes between samples and over time. This variation was essential to assess whether the Magritek Kea device was sensitive enough to detect changes in meat quality traits between samples and over time.

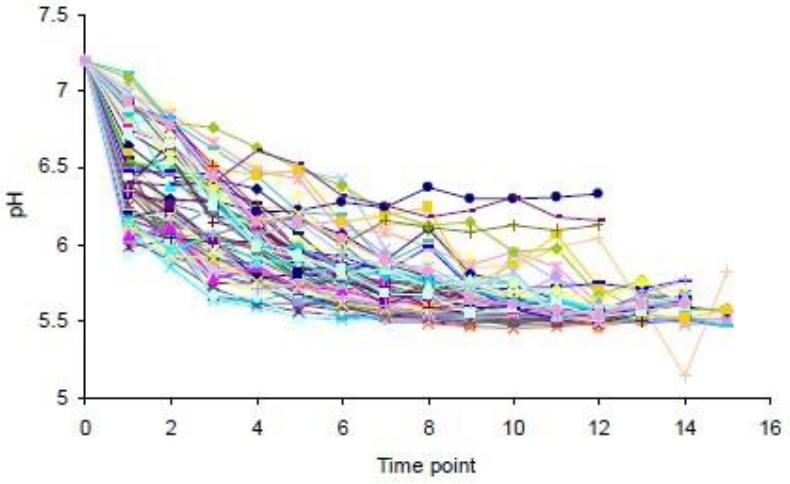

Figure 2. pH versus time point for all samples

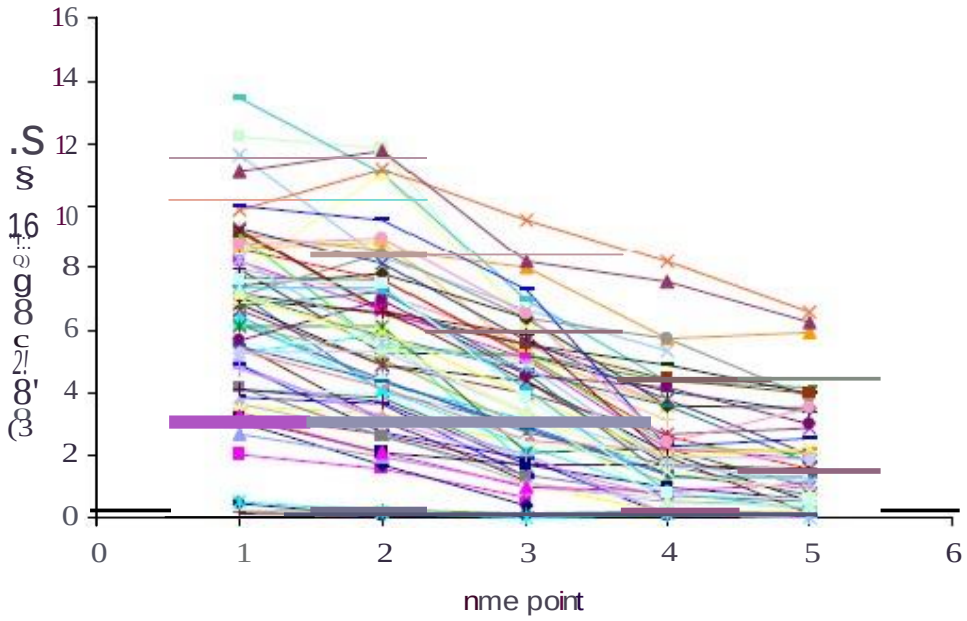

Figure 3. Glycogen concentration versus time point for all samples

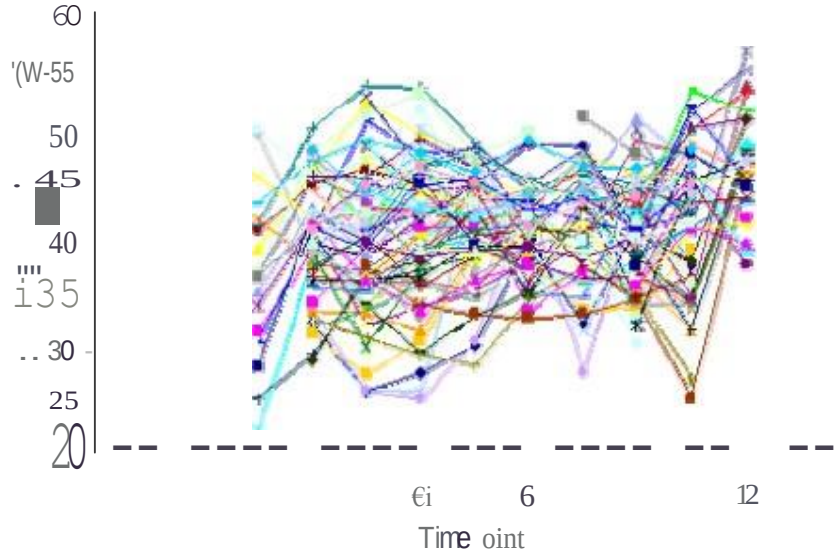

Figure 4. Water holding capacity versus time point for all samples

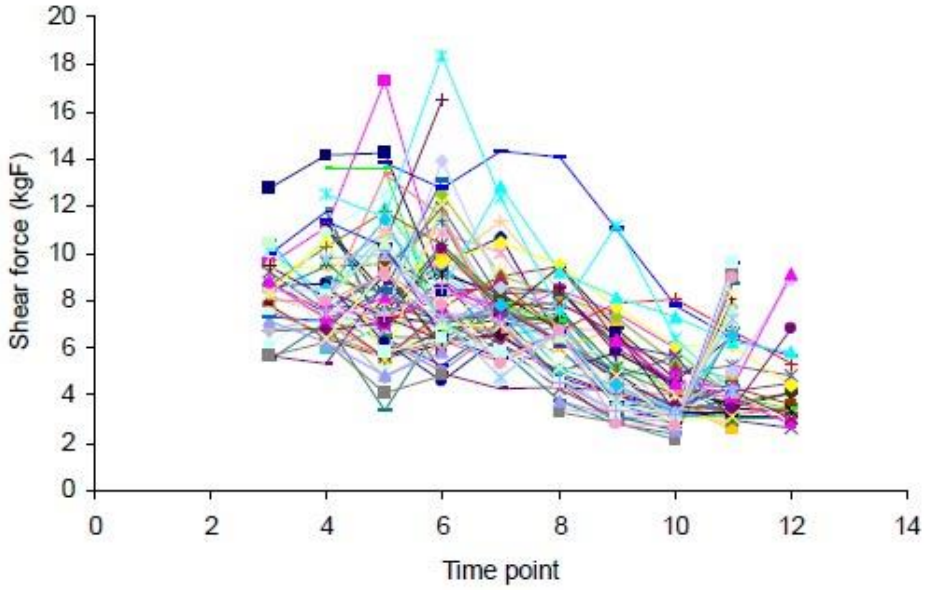

Figure 5. Shear force versus time point for all samples

3.2 Raw Data: LF-NMR relaxation curves

An example of the relaxation curves obtained for the four experiments are shown in Figure 6. The main observations from these curves were:

- "Experiment 1" (echo time of 0.14ms) did not monitor the full relaxation of the system. It ceased at 140ms compared to the other experiments that monitored the relaxation up to 320ms.
- The first points in the NMR curves appear to be spurious. The curves should start at a maximum amplitude and reduce over time. Therefore, the first points were removed from all experiments prior to exponential fitting.

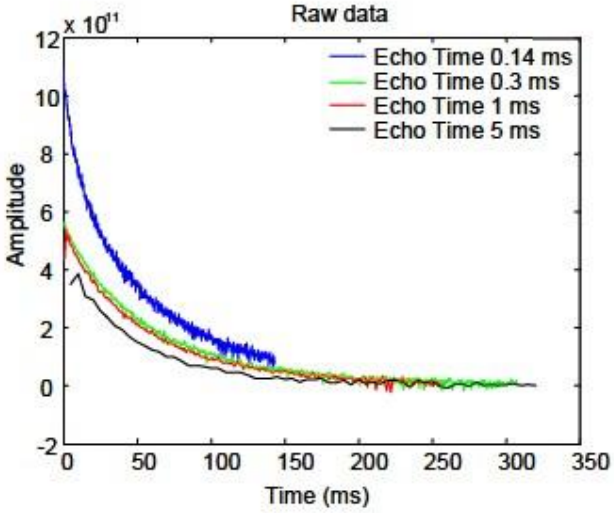

Figure 6. Raw relaxation curves from four CPMG experiments on a sample.

3.3 Data analysis: Trends over time and individual differences between samples

A principal components analysis (PCA, see Appendices 1 and 2 for results and definition) was conducted on mean centred relaxation curves to determine if there were any trends in the LF-NMR data over time. If trends were present over time then this may have related to changes in meat quality attributes, some of which also changed with time. However, no clear trends over time were observed in the PCA.

In order to correlate against specific meat quality attributes a partial least square (PLS) regression was carried out. PLS against water holding capacity, tenderness, glycogen content, pH and temperature were performed on the all LF-NMR data at the same time. The decay curves from the four experiments were concatenated (after removal of first points from the curves) giving raw data as shown in Figure 7. The information in the four curves should be very similar, thus it could enhance data analysis to have it represented several times.

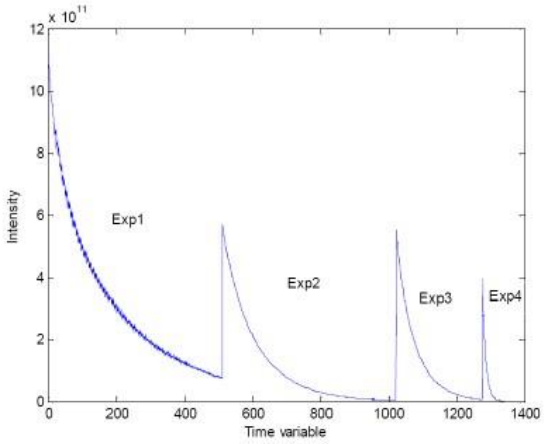

Figure 7. Decay curves (concatenated) from four CPMG experiments, which is how they were used in the PLS analysis.

The best correlation was obtained for water holding capacity with $r^2 = 0.19$. The r^2 values for the correlations with other meat quality traits are not detailed because they were below this value and not considered significant. Figure 8 shows the predicted water holding capacity using a PLS regression on the LF-NMR data against the measured water holding capacity. The model was based on 550 spectra of 1344 points (experiments 1-4 combined), (Time point 1+2 from kill group 2 was left out).

The reason for the very low correlations against water holding capacity, tenderness, glycogen content, pH and temperature was likely to be a low signal to noise ratio in the data. Figure 9 demonstrates that the regression coefficients resulting from the PLS across the same time scale as shown in Figure 7 (i.e. the results from all four experiments are concatenated) are quite noisy. If a high signal to noise ratio was achieved in the LF-NMR data (i.e. a good signal with low noise) then the curve in Figure 9 would be expected to be relatively smooth. The erratic nature of the curve in Figure 9 demonstrated that the LF-NMR data suffered from a low signal to noise ratio (i.e. too much noise to observe a clear signal).

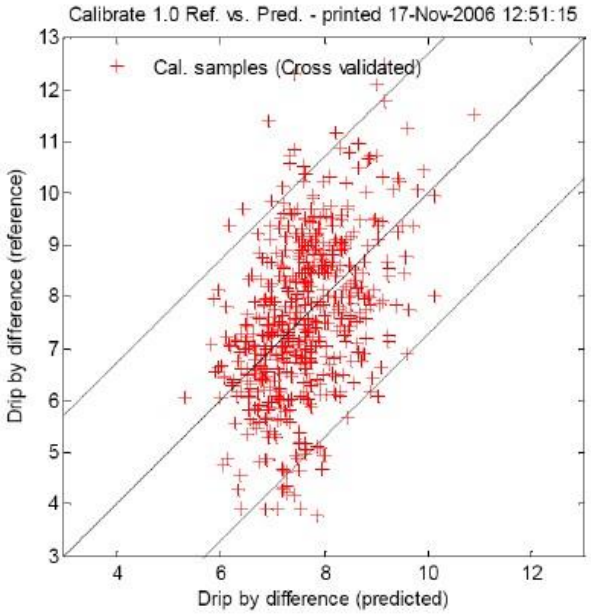

Figure 8. Predicted versus measured plot for water holding capacity predicted with LF-NMR

The reason for the very low correlations against water holding capacity, tenderness, glycogen content, pH and temperature was likely to be a low signal to noise ratio in the data. Figure 9 demonstrates that the regression coefficients resulting from the PLS across the same time scale as shown in Figure 7 (i.e. the results from all four experiments are concatenated) are quite noisy. If a high signal to noise ratio was achieved in the LF-NMR data (i.e. a good signal with low noise) then the curve in Figure 9 would be expected to be relatively smooth. The erratic nature of the curve in Figure 9 demonstrated that the LF-NMR data suffered from a low signal to noise ratio (i.e. too much noise to observe a clear signal).

The primary reason for this low signal to noise ratio was believed to be the lack of temperature control on the LF-NMR magnet. Any trends over time or individual differences between sample readings were "swamped" by the fluctuations in the signal thought to be caused by changes in temperature.

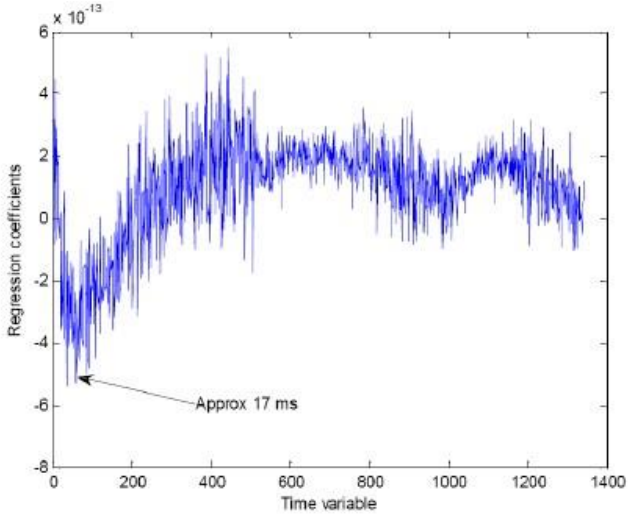

Figure 9. Regression coefficients (3 PLS components) for prediction of water holding capacity

3.4 Temperature Fluctuations

The fluctuation of temperature and B1 frequency was assessed by setting up an experiment where air temperature and B1 frequency were continuously measured over a two hour period. Figures 10 and 11 demonstrate that there was a decreasing trend in B1 frequency and there were fluctuations in both B1 frequency and air temperature over time.

Although the ambient air temperature was controlled in the room where the magnet was located, air temperature fluctuations of ±1°C (due to the on/off control of the air conditioner) appeared to significantly affect the B1 frequency and the LF-NMR readings. Additionally, the B1 frequency curve in Figure 10 demonstrates a "levelling out" of the B1 frequency at approximately 12:28 (Time). The device was left in the temperature controlled room during the entire experimental trial so this trend was not due to the magnet being brought into the room at a different temperature. However, it is possible that heat was generated by the LF-NMR device during operation, which warmed the magnet and this took some time to reach an equilibrium temperature with the air in the room. This suggests that better results may have been achieved if the instrument was operated until reaching an equilibrium temperature before making actual measurements. If the systematic errors due to temperature fluctuations are controlled then the precision of the LF-NMR measurements would naturally increase.

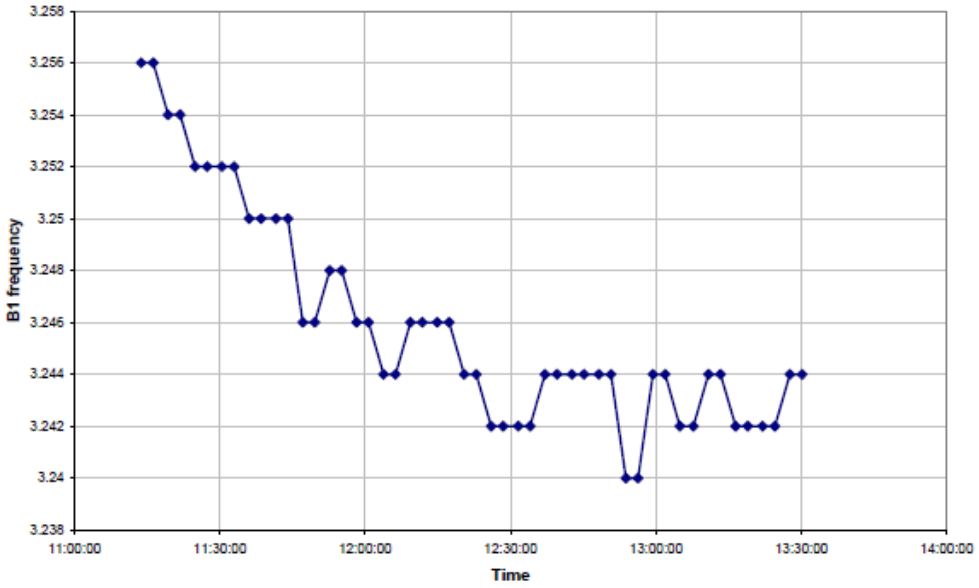

Figure 10. B1 frequency over time

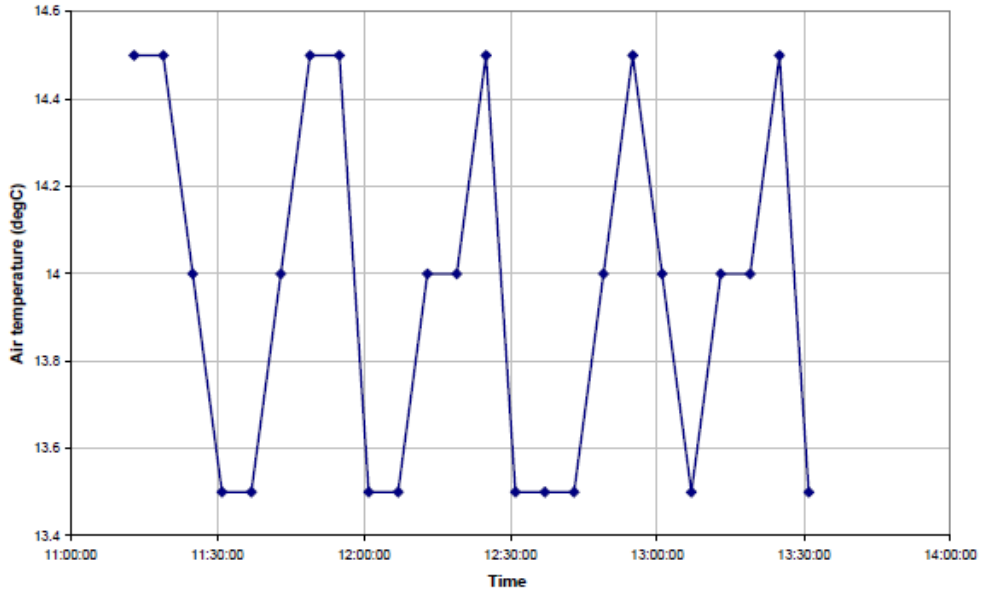

Figure 11. Air temperature in the room over time

Additionally, the temperature fluctuations would have caused changes in the B0 field, which would have also affected several aspects of the LF-NMR experiments such as initial magnetisation, precession frequency, RF tuning, and signal phase. The T2 relaxation time will have been affected in a seemingly random way due to the interactions of each of these LF-NMR parameters. For instance, one parameter may have increased the apparent T2 with decreasing frequency, whereas another parameter will have decreased T2 as the frequency drifted down. Some effects, in particular the

signal phase, have no correlation with frequency but will appear quite random depending on how the signal is averaged. The effect of these parameters in combination would be expected to result in random-like fluctuations in the data rather than any correlated trend of T2 with temperature or frequency.

To achieve greater temperature stability in the Kea device, Magritek have recently constructed a temperature stabilised probe which uses a circulating water system with heat exchange pipes embedded in the probe. Magritek are carrying out testing over next few months and will be ready to carry out further experiments with the improved system by the middle of 2007.

In addition, Magritek are currently working on other improvements that will give the instrument increased signal to noise ratio. These include:

(i) Prepolarising with a higher field. This will give the system a signal to noise ratio comparable to a higher field instrument
(ii) Shimming the gradient coils to improve the homogeneity. This will enable "fine-tuning" of the homogeneity, which will also increase the natural signal to noise ratio as smaller bandwidth acquisitions will decrease the noise in the measurement.
Both of these improvements are due to be completed and tested over the next few months.

3.5 Filtering noise: Trends over time

Measurements using benchtop LF-NMR instruments (23.2 MHz) have shown clear changes occurring at rigor. However, in this trial a PCA did not result in a trend with time.
In order to filter out the noise in the data and investigate trends over time, the LF-NMR data for stimulated (ES) and non-stimulated (NES) animals were aligned according to rigor time (determined as pH = 5.6). The data at each time point was then averaged. Figure 12 shows the trend in mean T2 relaxation time plotted against time from rigor.

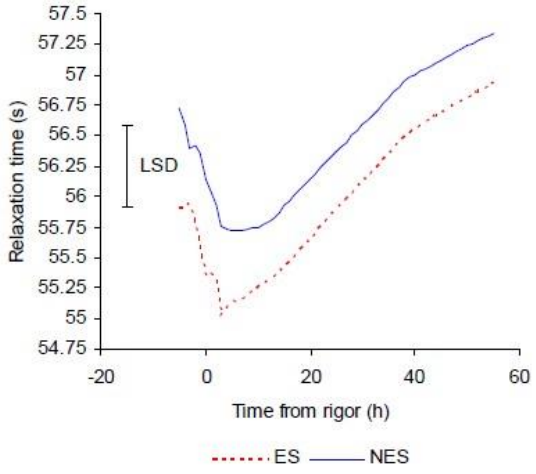

Figure 12 The effect of time from rigor on the mean T2 (experiment 2)

The trend in the mean data suggests that T2 relaxation time decreased to a minimum near rigor. The trend over time is likely to be valid despite temperature problems. This apparent change over time might be consistent with expected changes in extracellular water concentrations due to

rigor development and sarcomere shortening (Guignot *et al.*, 1993). However, the difference between the minimum and the maximum T2 mean values over time was relatively small being in the order of 4%. Furthermore the large variation between individual animal samples makes such an observation impossible on an individual animal basis. For such a measurement to be useful at the individual sample level the measurements would need to be more precise than was the case with the current data.

4 Conclusions

This experiment has found that further refinement of the Magritek one sided low field NMR instrument itself is necessary before such technology could be developed for an on-line meat quality application. A high degree of noise was found and this made it difficult to detect trends in LF-NMR data with time or any associations with meat quality data. Notwithstanding this there was some evidence of a change in mean T2 data over time. This finding was consistent with previously published studies using benchtop LF-NMR devices, indicating that there may be potential to use the Magritek devices if issues with signal to noise ratio can be solved.

Evidence was found that the high noise to signal ratio was at least partly due to the lack of temperature control in the NMR magnet. The impact of the temperature fluctuations was much worse than expected given that the measurements were conducted in a temperature controlled room. The B1 frequency was found to fluctuate between samples and in principle may have also fluctuated during measurements. Magritek is currently developing a new temperature stabilized probe and further research by the company is expected to indicate the success of this improvement.

4.1 Future implications and research by Magritek

The newly developed temperature-stabilised probe is expected to have better precision and this would allow detection of smaller changes in meat quality. This new system will need to be developed. Other improvements include:
(i) Prepolarising with a higher field. This will give the system S/N comparable to a higher field instrument
(ii) Shimming gradient coils to improve the homogeneity. This will enable us to "fine-tune" the homogeneity which will also increase the natural signal to noise as smaller bandwidth acquisitions decrease the noise in the measurement

5 References

Bertram, H.C., Purslow, P.P., Andersen, H.J. (2002). Relationship between meat structure, water mobility and distribution - A Low-Field NMR study. *J. Agric. Food Chem.* 50(4): 824-829.

Bertram, H.C., Engelsen, S.B., Busk, H., Karlsson, A.H., Andersen, H.J. (2004). Water properties during cooking of pork studied by low-field NMR relaxation: effects of curing and the RN— gene. *Meat Science* 66: 437-446.

Devine, C.E., Wells, R.W., North, M.F. (2006). Variation of meat shear force measurements – a characteristic of meat. Proceedings 52nd International Congress of Meat Science and Technology, Dublin, 575-576.

Devine, C.E., Wells, R.W., Lowe, T.E. (2004). Factors affecting the water changes in meat during tenderisation. Proceedings 50th International Congress of Meat Science and Technology, Helsinki, 385-388.

Graafhuis, A.E., Honikel, K.O., Devine, C.E., Chrystall, B.B. (1991). Meat tenderness of different muscles cooked to different temperatures and assessed by different methods. Proceedings 37th International Congress of Meat Science and Technology, Kulmbach, 365-368.

Guignot, F., Vignon, X., Monin, G. (1993). Post mortem evolution of myofilament spacing and extracellular space in veal muscle. *Meat Science* 33: 333-347.

Bertram, H.C., Andersen, H.J., Karlsson, A.H. (2001). Comparative study of low-field NMR relaxation measurements and two traditional methods in the determination of water holding capacity of pork. *Meat Science* 57: 125-132.

Krisman, C.R. (1962). A Method for the Colorimetric Estimation of Glycogen with Iodine. *Analytical Biochemistry* 4: 17-23.

Micklander, E., Peshlov, B., Purslow, P.P., Engelsen, S.B. (2002). NMR cooking: monitoring the changes in meat during cooking by low-field 1H-NMR. *Trends in Food Science and Technology* 13: 341-346.

Micklander, E., Bertram, H.C., Marnø, H., Bak, L.S., Andersen, H.J., Engelsen, S.B., Nørgaard, L. (2005). Testing of an on-line ultrasound method for early discrimination of high and low water-holding capacity in pig carcasses – a study including LF-NMR. *Food Science and Technology* 38: 437-445.

Tornberg, E., Wahlgren, N.M., Brøndum, J., Engelsen, S.B. (2000). Pre-rigor conditions in beef under varying temperature- and pH-falls studies with rigorometer, NMR and NIR. *Food Chemistry* 69: 407-418.

Van Oeckel, M.J., Warnants, N., Boucqué, Ch.V. (1999). Comparison of different methods for measuring water holding capacity and juiciness of pork versus on-line screening methods. *Meat Science* 51: 313-320.

Wahlgren, N.M., Tornberg, E. (1996). Ageing of beef studies by using different instrumental techniques and sensory tenderness Proceedings 42nd International Congress of Meat Science and Technology, Lillehamer, 432-433.

Wahlgren, N.M. Devine, C.E. and Tornberg, E. (1997). The influence of different pH-courses during rigor development on beef tenderness. Proceedings 43rd International Congress of Meat Science and Technology, Auckland, G1-37 622-623.