final report

Project code: A.MQT.0027

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Date submitted: October 2007

Date published: March 2011

PUBLISHED BY
Meat & Livestock Australia Limited
Locked Bag 991

Preliminary study of a Magritek Halbach NMR instrument as a device for shear force and drip loss measurement in meat

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government to support the research and development detailed in this publication.

Summary

• Nuclear magnetic resonance (NMR) technology has been used to study meat quality primarily in research applications. Developments by the New Zealand company Magritek offers the possibility that low field (LF) NMR technology could be suitable for commercial “on-line” applications.

• This report details an experiment conducted collaboratively by AgResearch MIRINZ, Murdoch University and Magritek at Magritek, Wellington, New Zealand. The aim of the experiment was to examine the signal from a Halbach NMR instrument and how it correlates to shear force and drip loss. A previous trial on a limited number of samples suggested that the Halbach NMR instrument could predict shear force during ageing. The present trial was carried out on a larger sample set to verify the encouraging result.

• A two-factorial experiment was designed using electrical stimulation and wrapping to create a sample set of lamb loins (n = 40) that varied in meat tenderness. Shear force and drip loss measured day 1 to 4 post slaughter were compared to LF-NMR data.

• Relaxation measurements were significantly affected by ageing when the same sample was measured in the Halbach NMR instrument on day 1 to day 4 post slaughter. The T_{21} time constant decreased, the T_{21} population increased and the T_{22} population decreased over the ageing period. This indicates an increased concentration of water within the intramyofibrillar space and decreased concentration in the extramyofibrillar space facilitated by proteolysis and concomitant swelling of the muscle fibers.

• The overall correlation between shear force and NMR relaxation measurements was 0.62 explaining 69% of the variation. Models fitted for the four different ageing times resulted in individual R^2 of 0.40 to 0.84. The correlation may be improved if the effect of within muscle variability is reduced by using the same sample for both measurements. This is possible with a one-sided NMR instrument like the Magritek NMR MOLE.

• This research indicates that increasing T_{21} population (K_{22}) and a decreasing T_{21} time constant is associated with more tender meat. The opposite result has been observed in pork. This result needs to be further confirmed with red meats.

• The prediction for drip loss gave a lower value of R^2 (0.42 explaining 50% of the variation). Further work will utilise the centrifuge drip loss method and as a result stronger correlations are expected.

• The correlation between shear force and the NMR relaxation parameters is a positive result, providing further evidence to support ongoing research using NMR to predict tenderness online in *post rigor* meat. Further work with the Halbach and an upgraded one-sided NMR instrument like the Magritek NMR MOLE is recommended to confirm and improve the correlations.

Contents

1. Introduction

Water within meat is predominantly contained (about 85%) in the intramyofibrillar (or intracellular) space (Huff-Lonergan et al. 2005) with the remainder (15%) being located in the extramyofibrillar (or extracellular) space (Hamm 1975; Lawrie 1998). Most of the intramyofibrillar water is considered to be immobilised water which is held by stearic forces between the thick and thin filaments of the myofibril (Honikel *et al.* 1986). This water is not able to freely flow from the tissue as it is held tightly in the structure of the myofibrils. Within meat other water populations also exist: (1) Bound water which is tightly bound to proteins which is unmovable even after physical disruption or cooking and (2) Free water which can easily be removed with little physical force and believed to reside predominantly in the extramyofibrillar space.

The relative location of water within the muscle depends on factors such as pH, sarcomere shortening and protein characteristics (Huff-Lonergan *et al.* 2005). During the conversion of muscle to meat there is both a longitudinal and lateral contraction of the muscle fibre and altered membrane and structural properties (Pearce and Rosenvold 2007). This contraction of the muscle fibre essentially 'squeezes' water out the immobilised water from between the myofibrils into the extramyofibrillar space (Kristensen *et al.* 2001; Offer *et al.* 1988). The subsequent process of proteolysis results in the degradation of cytoskeletal proteins such as desmin and cell adhesion molecules such as integin, weakening of the myofibrils and will affect water distribution. The reduced myofibrillar strain on the cell membrane results in an inflow of extracellular water to the muscle cell and the swelling of the myofibrillar space (Kristensen *et al.* 2001; Melody *et al.* 2004; Offer *et al.* 1988).

There are a number of consequences of water movement in meat that can affect meat quality. Increased transfer of water into the extracellular space during the conversion of muscle to meat is associated with greater drip loss (Guignot *et al.* 1993). Greater desmin degradation will reduce the shrinkage of the muscle cell and allow an inflow of extracellular water to the muscle cell, swelling of the myofibrillar space and increase water holding capacity and potentially tenderness (Pearce and Rosenvold 2007). Thus the evaluation of water states within the muscle may provide an important strategy to evaluate meat quality. On the other hand this represents a challenge since the preferred measurement method is non-invasive.

Nuclear Magnetic Resonance (NMR) has been extensively used in meat science allowing the evaluation of complex protein structures as well as the different kinds of water interaction within these systems (Bertram *et al.* 2004; Bertram *et al.* 2001; Bertram *et al.* 2002b; Bertram *et al.* 2007; Sorland *et al.* 2004; Yan *et al.* 1996). Among several approaches, NMR relaxation measurements have proven to be useful on the evaluation of water state in the muscle (Bertram *et al.* 2002b), where the major feature is the differentiation between water population and mobility within muscle microstructure.

The NMR T_2 relaxation of the water of muscle and meat has been described as non-mono-exponential which can be separated into the relaxation decay of two or three exponential populations, representing the concentration of the inherent water to these populations. These exponential components represent two to three major water

compartments in meat. The major population, T_{21}, is characterized by a fast time constant of 30-50 ms and contributes 80-95% of the relaxation of T_2. The second and slower population, T_{22}, has a time constant of 100-250 ms and accounts for 5-15% (Bertram *et al.* 2004). The T_{21} time constant is more likely to reflect water located within highly organized protein structures, e.g. water in tertiary and quaternary protein structures and spatial with high myofibrillar protein densities including actin and myosin filament structures (intramyofibrillar), while the T_{22} time constant reflects all the water located outside the myofibrillar network (extra-myofibrillar). Within meat, any change in the T_{21} time constant implies alterations in the structural organisation of myofibrillar water. Whereas the T_{22} time constant represents the *post mortem* reorganization of water closely associated with changes in membrane properties (Bertram *et al.* 2002b).

The development of open side Low Field (LF) NMR probes by NZ based Magritek has created the possibility for this measurement equipment to be used non-invasively in process monitoring of meat processing. Ideally the instrument will reveal changes in meat quality over time that will contribute significant scientific knowledge but also be able to detect differences in tenderness, essential for an on-line grading system. The prototype LF-NMR systems has the potential to manage quality during processing and predict product performance in the market as well as providing robust data which can be fed back to farmers and used in decision support.

In a previous study, relaxation measurements performed in a Halbach NMR instrument showed correlation with shear force (R^2 = 0.61), but in a limited number of samples (Rosenvold et al., 2007). However, the Halbach NMR instrument is not an open-top instrument; small meat samples must be excised prior to measurements.

The aim of the experiment described in this report was to investigate the ability of the Halbach NMR instrument to predict meat tenderness and determine its correlation with drip loss.

2. Methodology

2.1 Animal and treatments

To test the Halbach NMR instrument a sample set of lamb *M. Longissimus dorsi* (LD) with variation in tenderness was created through electrical stimulation (stimulation (SS)/no stimulation (US)), wrapping (WW/UW) and ageing time (1 to 4 days post slaughter) using a factorial design (see Table 1).

A total of 40 LDs from 20 lambs were included in this experiment. The lambs were slaughtered at the Ruakura abattoir using either a captive bolt or electrical headstunner for stunning (see Table 5). After dressing and immediately prior to electrical stimulation, the US LDs were removed from the carcasses, the carcasses were then electrically stimulated (80 V peak, 14.28 pulses s^{-1} for 30 seconds) and the SS LDs were removed from the carcasses.

When all 40 LDs were removed from the carcasses half of the LDs were tightly wrapped in four layers of cling film. The LDs were then taken to the laboratory where they were placed in waterproof polyethylene bags and immersed in a water bath at 35°C until rigor (defined as the time point when the pH fell below 5.6 for normal pH muscle or when the pH ceased to fall for muscles with elevated ultimate pH values) was reached.

2.2 Samples

When the LDs reached 5.6, the two LDs per animal were cut in half and allocated as sample A, B, C and D (Table 5). Halbach NMR measurements, shear force and drip measurements were measured repeatedly on day 1 (sample A), day 2 (sample B), day 3 (sample C) and day 4 (sample D).

After taking a sample for sarcomere length measurements, the LDs were placed in an ice bath until the next morning. The next morning the LDs were placed in an esky at ambient temperature and transported to Magritek, Wellington, where the NMR measurements took place. At Magritek the samples were kept at room temperature.

2.3 Meat quality measurements

The samples were 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.

For drip loss, a meat sample from each animal (~0.5 g for each of three replicates) was removed for measurement of drip by a filter paper press method (Rosenvold et al., 2007).

Table 1 Treatment allocation: The two loins from each animal (n = 20) were allocated to electrical stimulation (stimulation (SS)/no stimulation (US)) and wrapping (WW/UW). The lambs were stunned by captive bolt (CB) or electrical stunner (ES).

Animal ID (stunning method)	Unstimulated (US)			Stimulated (SS)	
		Wrapped (WW)	Unwrapped (UW)	Wrapped (WW)	Unwrapped (UW)
1 (CB)		X			
2 (CB)			X		
3 (CB)	X				
4 (CB)				X	
5 (CB)		X			
6 (CB)			X		
7 (ES)			X		
8 (CB)		X			
9 (ES)				X	
10 (CB)	X				
11 (ES)				X	
12 (ES)	X				
13 (ES)			X		
14 (ES)	X				
15 (ES)				X	
16 (CB)		X			
17 (ES)				X	
18 (CB)		X			
19 (ES)			X		
20 (CB)	X				

2.4 LF-NMR measurements on the Halbach NMR instrument

Four sub-samples of approximately 1 x 1 x 4 cm^3 were excised from each sample. Two of the sub-samples were cut in parallel (sub-sample 1 and 2) and two were cut perpendicular to fibre direction (sub-sample 3 and 4). The samples were placed in 5 mL tubes and measured in the Halbach NMR instrument in the tubes.

Sub-samples from the A sample were measured each day (1 to 4 post slaughter) using the exact same sub-samples each day. On days 2-4 the four sub-samples were dried and reweighed and placed in new clean, dry tubes. The four sub-samples from B, C and D samples were measured only on days 2, 3 and 4, respectively.

Each sub-sample was placed in the Halbach NMR instrument. When the four sub-samples were measured the procedure was repeated giving a total of 8 replicates per animal at each time point. LF-NMR measurements were carried out using Carr–Purcell–Meiboom–Gill (CPMG) sequence with settings described in Table 2. A duplicate vegetable oil measurement was taken prior to the start and regularly throughout the trial. The oil sample was measured in duplicate.

Drip loss analyses were carried out (in triplicate) every day and samples for shear force analysis were frozen using the A, B, C and D samples on days 1, 2, 3 and 4, respectively.

Table 2 - Parameters for Carr–Purcell–Meiboom–Gill (CPMG) sequence for the Halbach NMR instrument.

B1 frequency (MHz)	11.752 - 11.821
Repetition Time (ms)	1500
90 Amplitude (db)	-11
180 Amplitude (db)	-17
Pulse length (µs)	15
Echo Time(µs)	200
Number of Echos	512

*B1 frequency is sample dependent

2.5 Data analysis and statistical approach

The dynamics of molecules and their local environments can be evaluated with proton NMR relaxation times (T_1 and T_2). The T_2 transverse relaxation time, in general terms, is related to the relaxation curve by equation (1) (Goelman, et al 1995, Hürlimann et al 2001).

$$y = E(t, T_{2,j}, k_j) = \sum_{j=1}^{m} k_j e^{\left(\frac{-t}{T_{2,j}}\right)} \quad (1)$$

where *y* corresponds to amplitude of the signal at the echo time *t*, $T_{2,j}$ is the relaxation time constant for the species *j* and k_j the proportion of specie *j* in the signal (also designed as population of *j*).

The NMR relaxation curves were normalised to obtain the maximum amplitude equal to 1. The parameters, $T_{2,j}$ and k_j, of bi-exponential curves were estimated by non-linear least square in R language (www.r-project.org). This method determines the nonlinear (weighted) least-squares estimates of the parameters, based on a Gauss-Newton algorithm (*see* http://netlib.bell-labs.com/netlib/port/ for the algorithm documentation). This code was chosen due its absolute criterion, which does not declare convergence to false optima (Graves, 2003).

Generalized additive models (GAMs), with integrated smoothness estimation and thin plate regression spline, were used to estimate the relationship between shear force or drip and NMR parameters, $T_{2,j}$ and k_j. For GAMs, the package “mgcv” in R language (www.r-project.org) was used (Wood, 2000).

3. Results and discussion

3.1 Raw data: Shear force and drip loss

The measurements of shear force are presented in Table 3 and Figure 1. Figure 1 also presents the error associated to shear force measurements. The drip loss is summarized in the Table 4.

Table 3 – Shear force measured in LD day 1 to day 4 post slaughter. Average, minimum, maximum and standard deviation is shown.

Days post slaughter	1	2	3	4
Average	9.8	7.7	6.8	6.0
Max	17.8	21.4	13.9	16.6
Min	3.5	2.2	1.6	2.4
Std	3.5	4.1	3.0	3.1

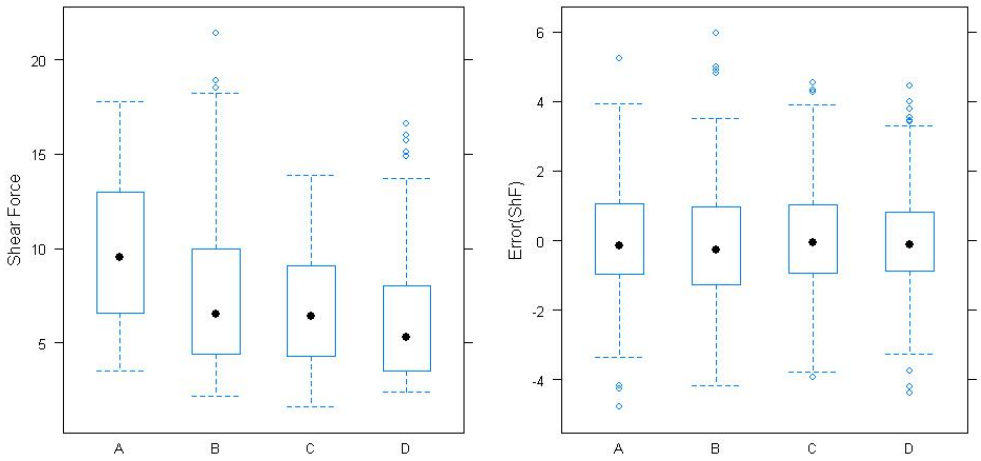

Figure 1.
(a) Shear force distribution for samples measured day 1 to day 4 post slaughter (A = day 1, B = day 2, C = day 3 and D = day 4). (b) Error, 'Error(ShF)', in the shear force measurements measured day 1 to day 4 post slaughter (A = day 1, B = day 2, C = day 3 and D = day 4). For further data on the error refer to Figure 11, APPENDIX A.

Table 4– Drip loss measured in LD day 1 to day 4 post slaughter. Average, minimum, maximum and standard deviation is shown.

Days post slaughter	1	2	3	4
Average	20.7	20.6	20.3	21.0
Max	21.8	22.6	22.3	23.0
Min	18.4	19.3	17.2	19.1
Std	0.9	0.9	1.5	1.2

3.2 Data analysis

Ageing effect

The A samples was measured daily from day 1 to day 4 post slaughter. The NMR results (Figure 2) showed a significant decrease in the fast T_{21} time constant with the T_{22} time constant remaining constant with ageing time. The T_{21} time constant was between 30-50 ms and the T_{22} was between 100 and 140 ms. There were also changes in the K_{21} and K_{22} which represent the proportion of water represented by either the T_{21} or T_{22} time constants. An increased proportion reflects an increase in the size of the population. The K_{21}, which represents the portion represented by the T_{21} time constant, increased with ageing time. The K_{22} was reduced with ageing time.

Previous research has suggested that the T_{21} time constant represents the intramyofibrillar water population essentially the immobilized water population with a T_{21} time constant of 30-50 ms with a T_{21} population of 80-95% and the T_{22} represents the extramyofibrillar or free water population with a T_{22} time constant of 100-250 ms and T_{22} population of 5-15% (Bertram & Andersen, 2004). Similar time constants and populations were observed in this experiment so we are detecting similar water populations, confirming the performance of the Halbach NMR instrument.

To understand the results and implications it is essential to understand that any change in the T_{21} time constant and population implies alterations in the structural organisation of intramyofibrillar water. Whereas the T_{22} time constant represents the *post mortem* reorganization of water closely associated with changes in membrane properties (Bertram *et al.* 2002b). Any increase in population represents an increase in the number of protons (or water) in an area. Furthermore, a decreasing in the T_{21} or T_{22} time constants reflects an increased probability of a water molecule meeting a surface which acts as a relaxation sink either due to a decreased area containing the proton or due to an increased concentration of protons.

An increased T_{21} population (increase in K_{21}) over the ageing period indicates an increase in the number of protons in the intramyofibrillar space over the ageing period. A decrease in the T_{21} time constant is occurring because there is an increase in the number of relaxation sinks within the myofibrils, i.e. protons. In other words this result indicates either an increasing uptake of water into the intramyofibrillar space or alternatively/in addition the release of water from bound proteins during the proteolysis process (Pearce and Rosenvold 2007). The decrease in the T_{22} population (K_{22}) demonstrates a decrease in water in the extramyofibrillar space. This is occurring for two reasons: some water is flowing from the extramyofibrillar into the intramyofibrillar space but a larger portion is being lost as drip. There was a decrease in the weight of the dried A sample of between 0.08 to 0.2 g each day from samples roughly 2.5- 3 g in weight. Bertram *et al.* (2002a) demonstrated that the T_{22} time constant decreased proportionally with the amount of expelled water.

The lack of change in T_{22} time constant in conjunction with a decreased T_{22} population is an interesting result, possibly suggesting a decreasing volume in conjunction with a decreasing population of protons. Had the volume stayed constant in the presence of a decreasing population then the relaxation time should have increased. Instead the co-committal breakdown of membranes during this time may be resulting in less area in the extramyofibrillar space rather then a decrease in area due to shrinkage per se.

The change in T_{21} with time is consistent with other research. Straadt *et al.* (2007) demonstrated a decrease in T_{21} time constant with ageing time which coincided with the swelling of the muscle fibres as shown by confocal laser scanning microscopy. The swelling of the myofibrils is enabled because the degradation of the cytoskeleton during ageing removes the inter-myofibrillar and costameric connections and thereby reducing or removing the linkage between the *rigor* induced lateral shrinkage of myofibrils and shrinkage of the whole muscle fibre. This degradation enables the swelling of the myofibrils so that the meat structure can retain/hold more water hence the increase in intramyofibrillar water and increase in the T_{21} population (K_{21}) (Huff-Lonergan *et al.* 2005; Kristensen *et al.* 2001; Melody *et al.* 2004).

Fjelkner-Modig *et al.* (1986) demonstrated a positive relationship between T_{21} time constant and tenderness in pork implying that low amount of intracellular water, i.e. shrunken myofibrils, and a high amount of extracellular water is favourable with regard to tenderness. The results in this experiment does not agree with this as increasing tenderness was associated with a decreasing T_{21} time constant hence a higher amount of intracellular water. Further studies are required in this area to understand the interaction. Definite correlations between drip loss measured using the bag method and relaxation parameters have been observed in other studies (Bertram *et al.* 2001; Bertram *et al.* 2002a) however the method of drip loss analysis (press drip) in this study does not appear to have been sufficiently accurate and future experiments will target this.

The ageing process was also evaluated through independent samples (A to D) measured day 1 to day 4 post slaughter. The results of these measurements did not show a significant effect of ageing time (Figure 3). This may have occurred because the ageing effect over time is confounded with effects of within animal variation due to across muscle variation and the side of carcass the muscle was extracted. Variation in the changes in T_{21} between animals (see Figure 11, APPENDIX A) demonstrated by variable responses in T_{21} relaxation time. Similar effect of within animal variation is observed in the reference measurements (shear force and drip, Figure 4). It is also relevant to note the significant effect of muscle fiber orientation, which was expected due to the anisotropic nature of NMR relaxation measurements (Navon et al, 2007).

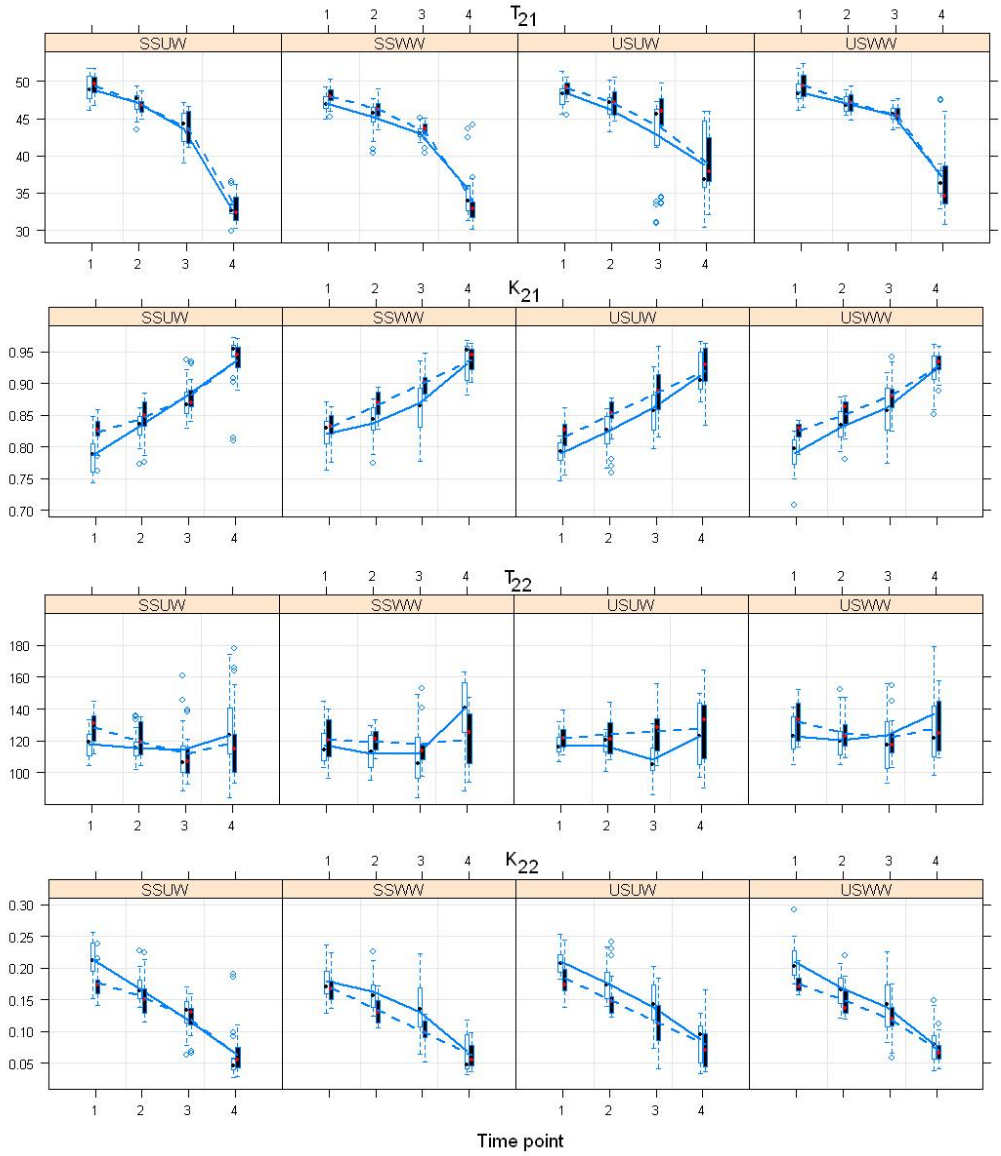

Figure 2
T_2 time constants and populations for sample A measured day 1 to day 4 post slaughter. Samples were either electrically stimulated and wrapped (SSWW), non-electrically stimulated and wrapped (USWW), electrically stimulated and unwrapped (SSUW) or non-electrically stimulated and unwrapped (USWW). Open bars corresponds to samples cut parallel to the fiber direction and full bars to samples cut perpendicular to the fiber direction. The relaxation times for T_{21} and T_{22} are in ms (10^{-3} seconds) and the populations K_{21} and K_{22} are a percentage of 100%

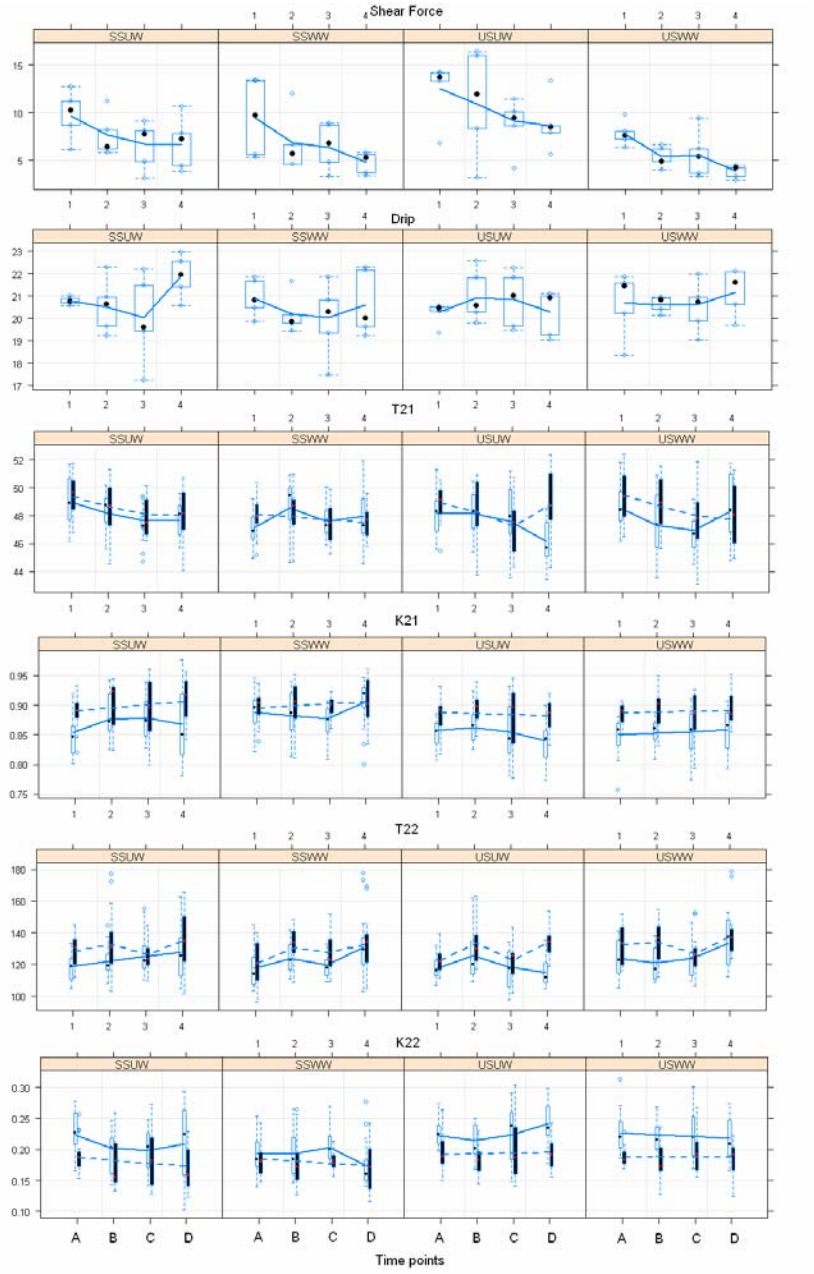

Figure 3
T_2 time constants and populations for samples measured day 1 to day 4 post slaughter (A = day 1, B = day 2, C = day 3 and D = day 4). Samples were electrically stimulated and wrapped (SSWW), non-electrically stimulated and wrapped (USWW), electrically stimulated and unwrapped (SSUW) or non-electrically stimulated and unwrapped (USWW). Open bars corresponds to samples cut parallel to the fiber direction and full bars to samples cut perpendicular to the fiber direction. The relaxation times are in ms (10^{-3} seconds) and populations are a percentage.

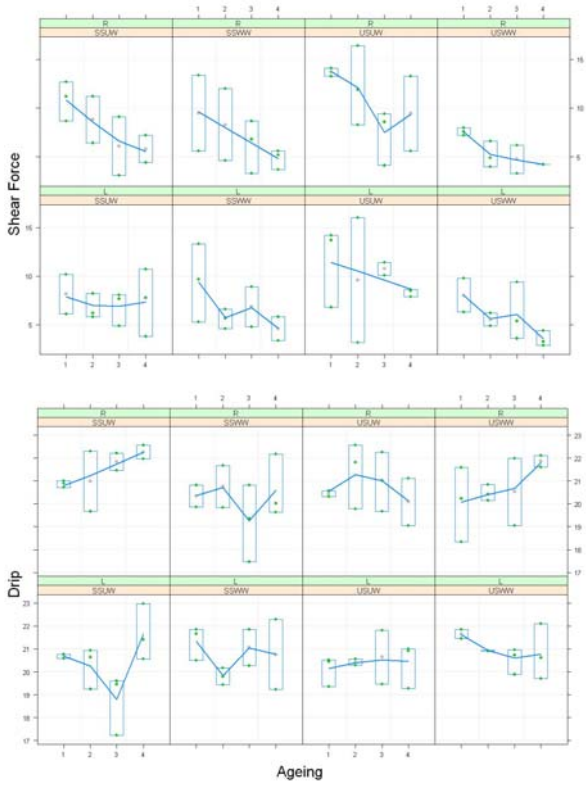

Figure 4
Shear force and drip measured day 1 to day 4 post slaughter (A = day 1, B = day 2, C = day 3 and D = day 4) taking into account the right (R) and left (L). Samples were electrically stimulated and wrapped (SSWW), non-electrically stimulated and wrapped (USWW), electrically stimulated and unwrapped (SSUW) or non-electrically stimulated and unwrapped (USWW).

A model for shear force

The analysis of ageing effect has shown the ability of NMR to detect changes in the post mortem muscle. It has also shown that variability within the muscle and fibre orientation significantly affects the relaxation measurements. The experiments were performed at room temperature, which changed through the day. Therefore, for every sample the B1 frequency, used to perform the relaxation measurements, was adjusted to compensate for temperature changes. Figure 5 shows the variability of the T_2 time constant and B1 frequency for the vegetable oil used as a reference. The T_2 time constant increased with time and decreased as function of the B1 frequency.

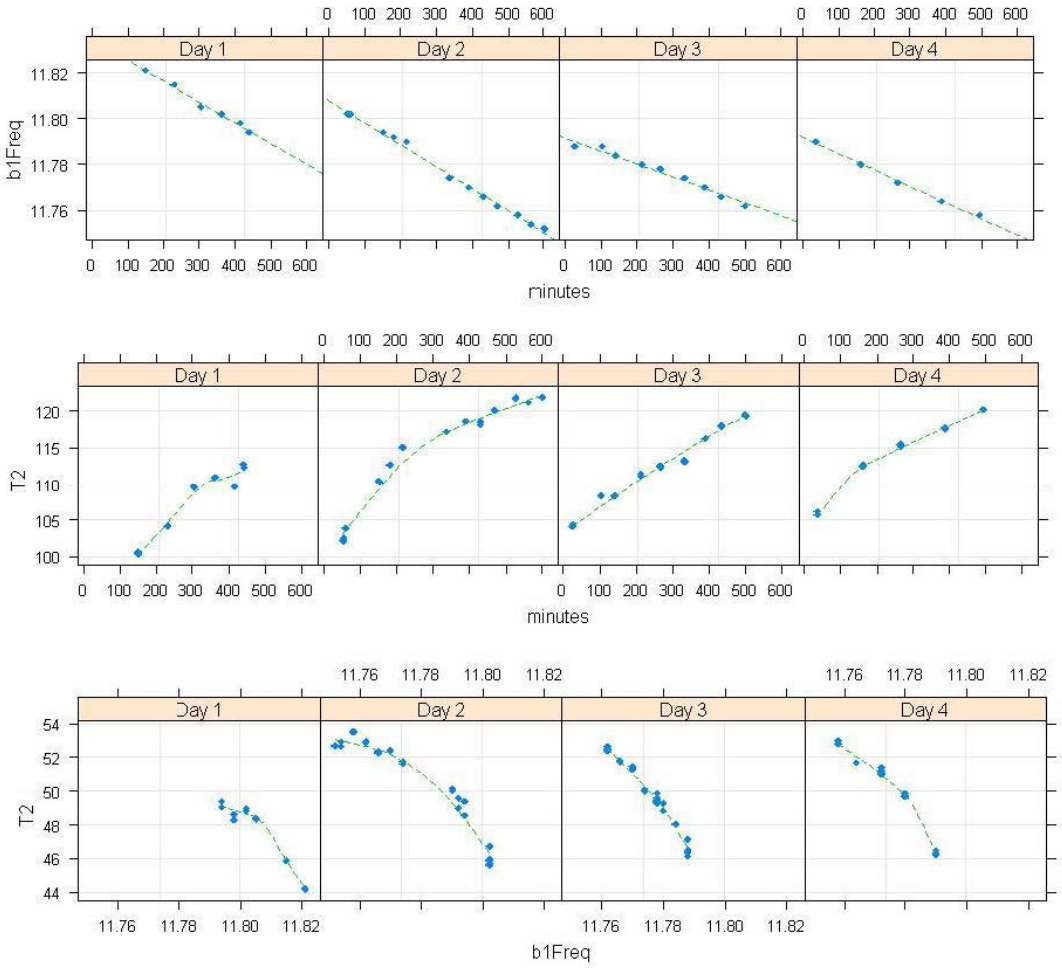

Figure 5
Relaxation measurements of vegetable oil used as reference. The time point 0 minutes for the first two plots corresponds to the first measurement performed in the day. The relaxation times are in ms (10^{-3} seconds).

The data presented in Figure 5 shows the fluctuation during the day, which significantly affects the B1 frequency and consequently the relaxation constants (T_2). As a result the complexity of the data increases. Any significant dependence between shear force and B1 frequency was investigated (Figure 6), none was found.

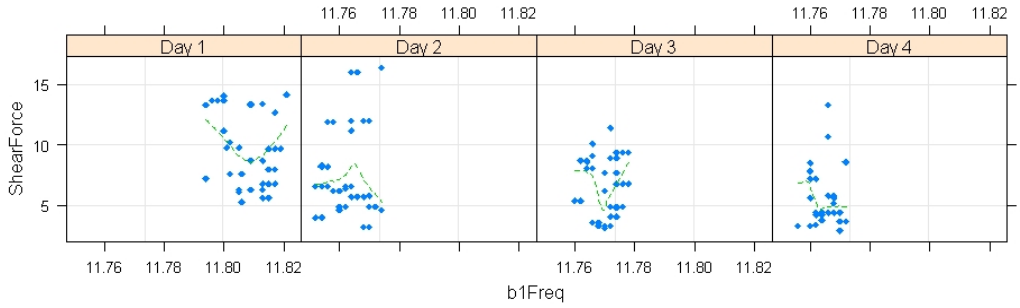

Figure 6
Covariation between B1 frequency and shear force.

Generalized Additive Models (GAM) were used to evaluate the correlation between shear force and the NMR relaxation measurements carried out on samples A to D. These models are based on thin plate regression spline and were chosen to accommodate the non-linear dependence between B1 frequency and T_2. In addition to relaxation time constants and population parameters, the ageing effect was also included in the model since shear force decreased with ageing time (Figure 2). The results from GAM model fitting are shown in Figure 7, which show a fit with R^2 equal to 0.62.

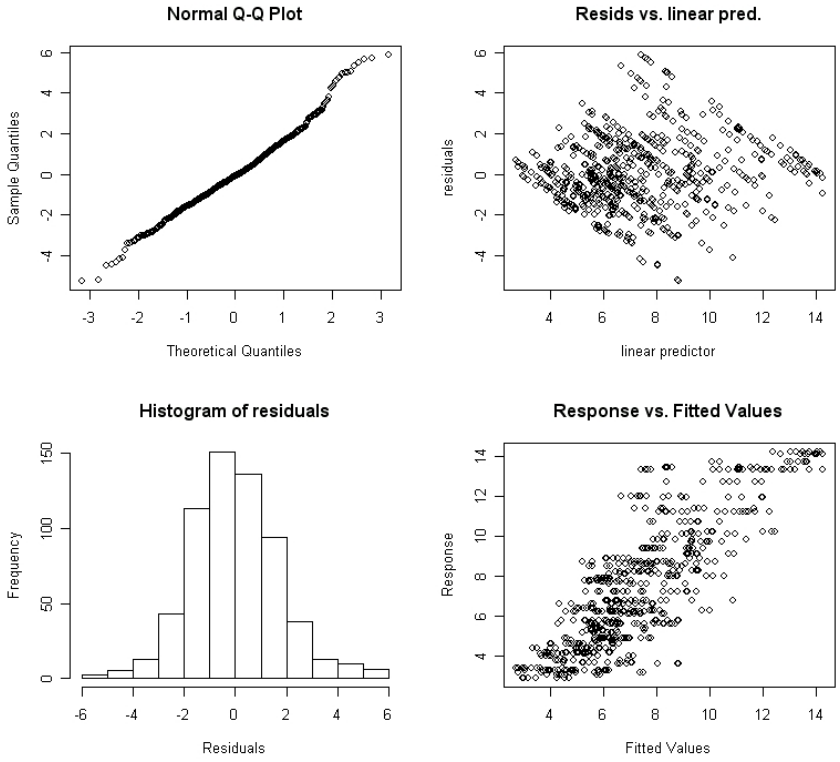

Figure 7

GAM model evaluation (R^2 = 0.62, Variance explained = 69%) used to predict shear force measured on samples A to D.

The results from GAM model decomposed in terms ageing and fibre orientation (Figure 8) show an improvement in the correlation between predicted and measured values within ageing time and fibre direction. The variation in shear force values decrease with ageing time as can be observed in Figure 1 and Figure 8, thus four independent models were fitted, one for each ageing time, and the results are plotted in Figure 9, where the correlation between shear force and the NMR parameters is still significant.

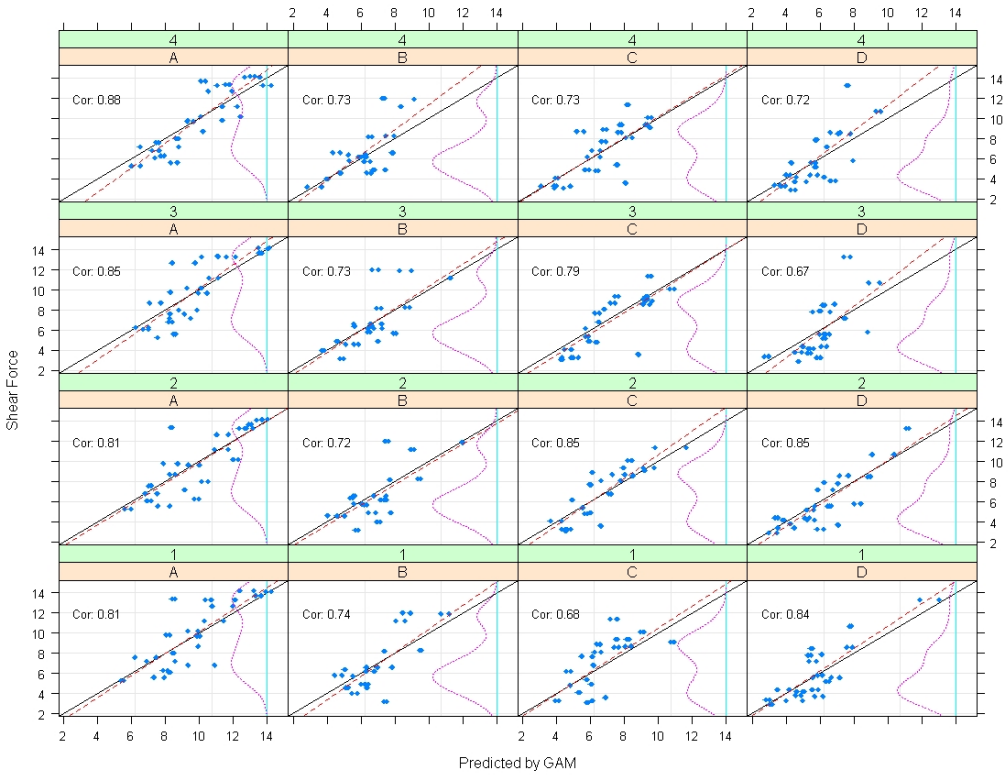

Figure 8

Predictions of shear force by a general GAM model (R^2: 0.62; explained variance = 69%) in samples measured day 1 to day 4 post slaughter (A = day 1, B = day 2, C = day 3 and D = day 4). 'Cor' is the correlation between predicted and expected values. The plot titles 1 to 4 are the four sub-samples with 1 and 2 being parallel to the fibre direction and 3 and 4 being perpendicular to fibre direction. The doted line in the right side of each model corresponds to density distribution of expected shear force values.

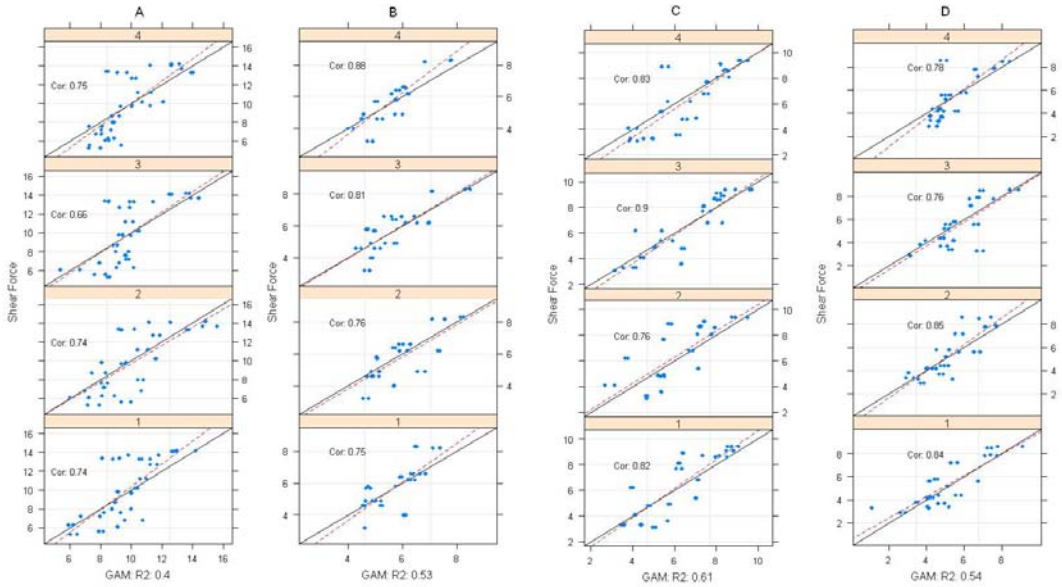

Figure 9

Predictions of shear force by GAM models independently fitted for samples measured day 1 to day 4 post slaughter (A = day 1, B = day 2, C = day 3 and D = day 4). 'Cor' is the correlation between predicted and measured shear force. The plot titles 1 to 4 are the four sub-samples with 1 and 2 being parallel to the fiber direction and 3 and 4 perpendicular to the fiber direction.

The predictions from GAM models for the global data, including all the samples in the model, have an overall R^2 of 0.62. The four models fitted independently for each ageing time have an R^2 between 0.40 and 0.61. In general terms, the reduction in R^2 may be due to the effect of confounding between ageing time and reduction in shear force. When the results are split in terms of fiber orientation, the correlation between measured and predicted values increased, compared to the overall R^2, this suggests an important effect of fiber orientation over the model.

A model for drip

The predictions for drip loss measured by the press drip method was based on a similar GAM model used for shear force including all samples are described in Figure 10, with an R^2 equal to 0.42. Similarly to shear force the drip press method gave a high variability in the reference measurement, which makes the model fitting a challenge.

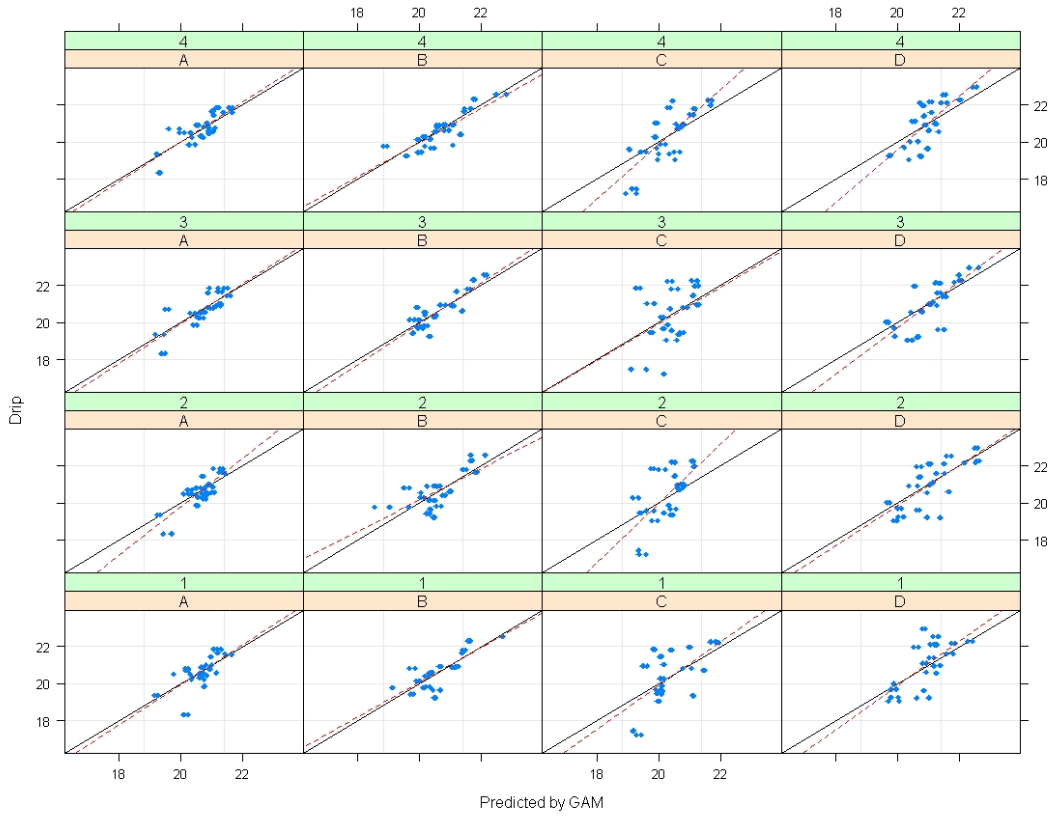

Figure 10

Predictions of drip loss by GAM models (R^2 = 0.42. Variance explained = 50%) independently fitted for samples measured day 1 to day 4 post slaughter (A = day 1, B = day 2, C = day 3 and D = day 4). 'C or' is the correlation between predicted and measured shear force. The plot titles 1 to 4 are the four sub samples with 1 and 2 being parallel to the fiber direction and 3 and 4 is perdendicular to the fiber direction.

4. Conclusions

A representative evaluation of LF-NMR relaxation measurements using a Magritek Halbach NMR instrument was performed on a lamb loin sample set which varied in tenderness and drip created through electrical stimulation, wrapping and muscle fiber orientation.

Ageing had a significant effect over relaxation measurements when the same sample was monitored day 1 to day 4 post slaughter. The T_{21} time constant decreased, the T_{21} population increased and the T_{22} population decreased over the ageing period. This indicates an increased concentration of water within the intramyofibrillar space and decreased concentration in the extramyofibrillar space. The muscle fibers are swelling with ageing period due to the degradation of desmin reducing the shrinkage of the muscle cell and allowing the influx of extramyofibrillar water into the intramyofibrillar space and swelling in size.

The overall correlation between shear force and NMR relaxation measurements was 0.62% explaining 69% of the variation in shear force. Models fitted for the four different ageing times resulted in individual R^2 between 0.40 and 0.84. The correlation with shear force was improved when the fiber direction of the sample was taken into the prediction model. This highlights the importance of anisotropic nature of NMR relaxation measurements in meat assessment.

This research indicates that increasing T_{21} population (K_{22}) and a decreasing T_{21} time constant is associated with more tender meat. An opposing relationship was observed in pork. This result needs to be further confirmed with red meats.

The prediction for drip loss gave a lower value of R^2 (0.42 explaining 50% of the variation in drip loss). Had the centrifuge drip loss method been used for this prediction may have been stronger and consistent with other studies which have shown high correlations. However, the centrifugation method could not be applied in the experiment as it was carried out at Magritek.

The major challenge in this study was the variability within muscle, since the sample measured by NMR is not the exact same portion of the muscle used to measure the shear force. This problem should be solved using a one-sided NMR instrument like the Magritek MOLE which provides the possibility of performing the relaxation measurement in the sample to be used for shear force measurement reducing the effect of within muscle variability.

5. Future implications

The obtained correlation – R2 = 0.62 explaining 69% of the variation - between shear force and NMR relaxation parameters is a positive result, providing further evidence to support ongoing research using NMR to predict tenderness online in *post rigor* meat. Further work with the Halbach and a one-sided NMR instrument like the Magritek NMR MOLE is recommended to confirm and improve the correlations. The NMR MOLE is currently being upgraded by Magritek to remove temperature fluctuation issues and improve the signal to noise ratio and further work will be conducted on this improved model when available in the early 2008.

The correlation between the Halbach NMR measurements and shear force can potentially be improved if the effect of within muscle variability is reduced by using the same sample for both measurements. This is possible with one-sided NMR instrument like the Magritek NMR MOLE.

The correlation to drip loss could be improved by the use of centrifugation methods rather then press drip and will be addressed in future work.

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APPENDIX A

Table 5: Treatment allocation: The two loins from each animal (n = 20) were allocated to electrical stimulation (stimulation (SS)/no stimulation (US)) and wrapping (WW/UW). The loins were divided in two and randomly distributed to samples A to D. RH = right loin, head end; RT = right loin, tail end; LH = left loin, head end: LT = left hand, tail end.

	Unstimulated (US)									Stimulated (SS)						
Animal	Wrapped (WW)				Unwrapped (UW)					Wrapped			Unwrapped (UW)			
	RH	RT	LH	LT	RH	RT	LH	LT	RH	RT	LH	LT	RH	RT	LH	LT
1					B	C	D	A								
2									C	D	A	B				
3	A	B	C	D												
4													D	A	B	C
5					C	D	A	B								
6									D	A	B	C				
7									A	B	C	D				
8					D	A	B	C								
9													A	B	C	D
1	B	C	D	A												
1													B	C	D	A
1	C	D	A	B												
1									B	C	D	A				
1	D	A	B	C												
1													C	D	A	B
1					A	B	C	D								
1													D	A	B	C
1					B	C	D	A								
1									C	D	A	B				
2	A	B	C	D												

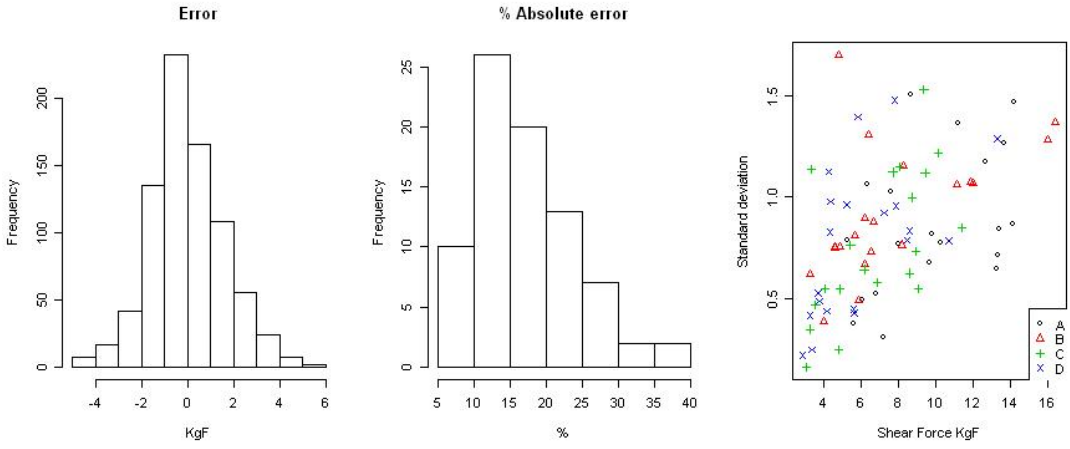

Figure 11

Error, 'Error(ShF)', in the shear force measurements measured day 1 to day 4 post slaughter (A = day 1, B = day 2, C = day 3 and D = day 4).

Figure 12

T_{21} time constants measured in samples A to D. The plots compare sub-samples measured in one fiber direction (1 = parallel to the fiber direction, 2 = perpendicular to the fiber direction) from the same LD (R = right, L = left). The relaxation times are in ms (10^{-3} seconds).