final report

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Prepared by: Dr Eric N Ponnampalam, Dr David L Hopkins,
Dr Leigh Ward, Dr Nigel Kirby and Professor Frank R Dunshea

Agriculture Research, Department of Economic Development, Jobs, Transport & Resources, Attwood, VIC 3049, Australia;

Department of Primary Industries, Centre for Red Meat and Sheep Development, PO Box 129, Cowra, NSW 2794, Australia;

The University of Queensland, St Lucia, Brisbane, QLD 4072, Australia;

Australian Synchrotron, 800 Blackburn Road, Clayton, VIC 3168, Australia;

Faculty of Veterinary and Agriculture Sciences, The University of Melbourne, Parkville, Vic. 3010, Australia;

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Enhancing meat measurement approaches

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Abstract

The small angle x-ray scattering (SAXS) synchrotron beamline and bioimpedance technologies were evaluated for their application to measure meat quality traits. Fifty topside (semimembranosus muscle: SM) and 50 loin (longissimus muscle: LL) equally collected from lamb and sheep carcases during autumn and spring seasons were used in this study. SM and LL muscle samples were measured with bioimpedance, which was followed by collection and processing of muscle samples for synchrotron analyses and traditional laboratory meat quality measures. Impedance data were influenced not only by the inherent chemical composition and hence the electrical properties of the tissue but also by the geometry of the sample under analysis. Measures are being sought that are independent of geometry, but provide indices of the compositional nature of the tissue. Currently, Cm, or a capacitive equivalent of resistivity, is the leading contender. At present impedance spectroscopy is most appropriately used to predict fat-free mass (lean) of tissues, for example lean content of shortloin in this study. However further research is required to test the practicality of this technology in predicting lean content of primal cuts or carcasses online in a commercial processing sector (abattoir). The results indicated that there was a strong positive relationship between the d-spacing of the actin/myosin fibril spacing (SAXS1 & SAXS2) and the cross sectional area of the hexagonal unit cell (Cell area) with the traditional measure of shear force evaluated on LL samples aged for either 1 or 5 days. There was also a strong positive relationship between intramuscular fat (IMF) content measured traditionally and the SAXS fat measure, with higher percentages of IMF resulting in higher values from the SAXS fat area measure. It was also found that there was an effect of position within the muscle on SAXS d-spacing measures (SAXS1, SAXS2 and Cell area) with samples collected from the cranial location of LL producing larger distances between muscle fibrils compared with samples collected at either the medial or caudal location. This study indicates that the SAXS technology could be a powerful research tool to examine structural components affecting tenderness of meat and fat content related to IMF affecting juiciness and flavour of meat. The results demonstrate that the SAXS and bioimpedance technologies could be powerful research tools in the future to determine not only the structural components of muscle, but also the composition of muscle relating to eating quality traits of meat. This work also indicates that these technologies have good potential, with adaption, for use in industry.

Executive Summary

Tenderness (texture), juiciness and flavour are the meat characteristics that indirectly influence consumer choice when purchasing lamb. Muscle fibre characteristics, intramuscular fat and collagen content are factors that affect the tenderness of meat while intramuscular fat or lipid content of muscle impacts on the juiciness and flavour of meat. Classical measures of traits like Warner Bratzler shear force, a proxy for tenderness, are destructive of samples and not applicable to on-line measurement. Related to this other traits such as intramuscular fat have remained elusive for on-line measurement. Bioimpedance and synchrotron are technologies that have the potential to address this area as applied to the measurement of carcass/muscle composition and meat eating quality traits. The question is can these technologies be focused on the measurement of muscle composition and/or meat quality traits?

Within the Australian lamb industry, the consistency and quality of lamb products has come under scrutiny, and the need to guarantee the eating quality of lamb and other sheep meat products has been identified. The introduction of technologies and research that provide information on meat quality aspects such as tenderness and intramuscular fat (IMF) content can help to increase processing efficiency and ensure a uniform product for retailers and consumers. Consumer demand has been the main driver for change from the subjective measurement of meat quality to the objective measurements currently used. Technologies have been adapted to carry out these objective measurements, with near infrared reflectance spectroscopy (NIR) at the forefront and promising technologies, such as Raman spectra, emerging. This project was to evaluate the application of bio-impedance and synchrotron (shortloin only) technologies on identifying the primal composition and muscle characteristics of shortloin and topside in lambs and sheep and comparing this against traditional laboratory methods.

In the current work the small angle x-ray scattering (SAXS) synchrotron beamline technology and bioimpedance technology were used in measuring the muscle components that influence meat quality traits. Fifty (50) lamb and 50 sheep carcases selected at a commercial abattoir from varying lots during autumn and spring seasons were used for this study. Fifty topside samples (semimembranosus muscle: SM) and 50 loin samples (longissimus muscle: LL) were collected at 18 hours post-slaughter in the boning room to determine meat quality traits. SM and LL muscle samples were measured with bioimpedance using an ImpediMed SFB7 bioimpedance spectrometer. This was followed by collection and processing of muscle samples for synchrotron analyses using the SAXS synchrotron beamline and traditional laboratory meat quality measures of tenderness (WB shear force), intramuscular fat (IMF), collagen content, sarcomere length (SL) and particle size analysis (PS). Synchrotron and bioimpedance data were processed and relationships with traditional meat quality measurements were derived using regression and/or correlation analyses.

Impedance data were influenced not only by the inherent chemical composition and hence the electrical properties of the tissue but also by the geometry of the sample under analysis. Measures are being sought that are independent of geometry, but provide indices of the compositional nature of the tissue. Currently, Cm, or a capacitive equivalent of resistivity, is the leading contender although novel data analytical approaches are being developed that may provide better tissue characterisation. At present impedance spectroscopy is most appropriately used to predict fat-free mass (lean) of tissues, for example lean content of shortloin in this study.

There were relationships between the SAXS measures and meat tenderness assessed by a traditional laboratory measure. The synchrotron data for myosin/actin characteristics (SAXS1

& SAXS2) and cell area both impacted on meat tenderness measured at 1 day ageing and in this case traditional meat quality measures such as particle size analysis (PS) and sarcomere length did not explain variation in shear force. Interestingly when SAXS1 and cell area were included in the model, the difference in tenderness between sheep versus lamb types disappeared. There was a strong relationship between intramuscular fat (IMF) and the fat area measured by the SAXS and this replaced GR and type of meat (sheep versus lamb) in the model. There was an effect of position of samples taken from a longissimus muscle for synchrotron analyses such that sample (slice A) collected from cranial location of the LL was consistently different from samples (slice B-D) collected from the medial and caudal location of the LL for the following traits (cell length, cell area and fat area). This indicates that there are structural and chemical differences (or changes) along the longissimus muscle, something that would have a bearing on the use of synchrotron data. The differences shown by SAXS measures between cranial and the medial/caudal positions of the LL were mirrored in shear force results, with the cranial section being more tender than the caudal section. The results show that the variation in the myofibrillar structure and fat distribution of muscle identified by synchrotron SAXS technology can be used for the detection of differences in IMF and tenderness of meat. The SAXS beamline presents a promising opportunity to determine meat toughness/tenderness and relative fat content and could be a useful experimental tool, overcoming the need for destructive sampling required for traits like shear force and IMF.

The findings of this study have shown that the SAXS and bioimpedance technologies could be powerful research tools in the future to determine not only the structural components of muscle, but also the composition of muscle relating to meat eating quality traits. The current study predicted the differences in actin/myosin spacing and fat distribution between positions within a muscle and between seasons. Future study is needed to evaluate the use of synchrotron technology in predicting components such as collagen, fat distribution or myosin/actin levels influencing eating quality traits (eg. tenderness, juiciness etc.) between muscles within an animal and between muscles from different growth stage of animals, which will provide valuable insight to improve eating quality aspects of lamb and beef. Furthermore, the current work indicates that bioimpedance technology is non-destructive procedure that could be automated and applicable online to predict fat-free mass or lean content of primal cuts or entire carcasses. However, further research is needed to test its application online.

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1 Background

Tenderness (texture), juiciness and flavour are the meat characteristics that indirectly influence consumer choice of purchasing lamb. Muscle fibre characteristics, intramuscular fat and collagen content are factors that affect tenderness of meat while intramuscular fat or lipid content of muscle impacts on juiciness and flavour of meat. These components in the muscle of growing animal may vary with animal age, nutritional background (i.e., finishing systems) and genetics. For example, increasing lean meat content in the carcass through on-farm finishing systems can negatively influence the tenderness (texture) of meat through a reduction in intramuscular fat in the muscle.

Within the Australian lamb industry, the consistency and quality of lamb products has come under scrutiny, and the need to guarantee the eating quality of lamb and other sheep meat products has been identified (Russell et al., 2005). The introduction of technologies and research that provide information on meat quality aspects such as tenderness and intramuscular fat (IMF) content can help to increase processing efficiency and ensure a uniform product for retailers and consumers (Lambe et al., 2009). Consumer demand has been the main driver for change from the subjective measurement of meat quality to the objective measurements currently used (Stanford et al., 1998). Technologies have been adapted to carry out these objective measurements, with near infrared reflectance spectroscopy (NIR) at the forefront (Lambe et al., 2009; Prieto et al., 2009) and promising technologies, such as Raman spectra, emerging (Schmidt et al., 2013).

Measurement of carcase composition and muscle characteristics is an ongoing area of investigation. Classical measures for traits like Warner Bratzler shear force a proxy for tenderness are destructive of samples and not applicable to on-line measurement. Related to this other traits such as intramuscular fat have remained elusive for on-line measurement. There are two technologies that have potential to address this area which require examination under controlled conditions. Bio-impedance is one such area, with major improvements in the capability of this technology as applied in the measurement of carcase or muscle composition. The question is can this technology be focused on the measurement of meat quality traits or composition?

The other technology is synchrotron small angle X-ray scattering (SAXS) beamline, which may be useful to differentiate muscle characteristics such as fibre type, collagen or muscle lipid factors that influence meat texture, juiciness and flavour. Again the question is can this scattering beamline technology be used to differentiate any meat quality traits further and relating it to traditional laboratory measures? For the first time this technology was used to identify the muscle characteristics of meat producing animals and relate these to meat quality aspects.

The SAXS and bioimpedance technologies could be used to produce information on the structure of the fibrils (actin, myosin and collagen), and potentially provide estimates of the intramuscular fat content and composition of muscle. This project was undertaken to evaluate the primal composition and muscle characteristics of shortloin and topside in lambs and sheep using bio-impedance and synchrotron SAXS (shortloin only) beamline technologies against traditional laboratory methods.

2 Projective Objectives

To examine the use of synchrotron technology and bioimpedance in differentiating the muscle characteristics (i.e. fibre thickness, collagen or fat distribution) that are associated with meat tenderness (i.e. texture).

3 Methodology

3.1 Carcass selection

Fifty lamb carcasses and the same number of sheep carcases (total n = 100) were used for this study. The muscle samples were collected in a commercial abattoir during autumn (March 2014; n = 50) and spring (October 2014; n = 50). The carcasses were selected in such a way so as to cover low, medium and high fatness ranges (see Fig. 1) from a range of lots in an attempt to provide a wide variation in fat and tenderness levels and on each occasion were selected over 2 slaughter days.

Fig. 1. Photo shows the range of lamb carcasses selected during autumn season 2014 for loin and topside muscle sample collection.

3.2 Carcass measurement and muscle sample collection

Carcass weight and GR fat depth were recorded. GR fatness was measured using a GR knife which was inserted into the carcass at the lateral surface of the twelfth rib, 110 mm from the midline and was recorded 15 h post slaughter. pH was measured using a pH meter/electrode (model TPS WP-80 pH meter with ionode probe IJ44c attached, TPS Pty Ltd., 4 Jamberoo Street, Springwood, Qld. 4127, Australia). It was calibrated using a buffer with known pH's of 4 and 7.

A 400-500g section of the m. *longissimus* (LL) (Product identification number HAM 4910; Anonymous 2005) was removed at 15 h post-mortem (PM) from the left side of each carcase, taking the muscle from the sacral lumbar junction through to the 8th rib. All external subcutaneous fat was removed from the LL muscles prior to sub-sampling. Firstly, bioimpedance measurements were performed from all LL muscle samples in the boning room at JBS abattoir. Then samples were collected for synchrotron measurement and other traditional meat quality assessments. Synchrotron measurements were conducted at the Australian Synchrotron facilities, Victoria, Australia. Four LL sub-samples (A-D) were then collected per carcase and subjected to measurement using the Small Angle X-ray Scattering (SAXS) beamline. A sub-sample of LL was prepared at 1 day PM for the measurement of Warner Bratzler (WB) shear force (SF1). Another sub-sample was aged at 4^{o}C for 5 days and measured for SF (SF5), while samples for particle size analysis (PS) were also aged for 1 and 5 days before being frozen for subsequent testing. Samples for sarcomere length (SL), intramuscular fat content (IMF) and collagen analysis were also taken at 1 day PM and

frozen until measurement. Sample collection for the various measurements is shown in Fig. 2.

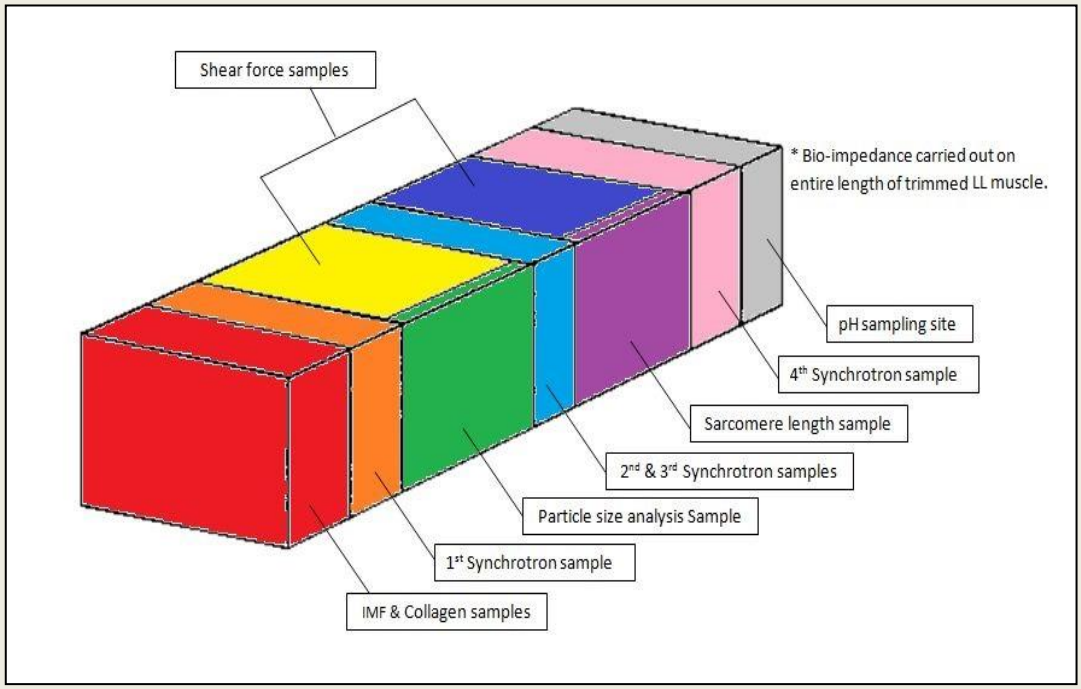

Fig. 2. Partitioning of LL muscle sampling

3.3 Bioimpedance measurements

Samples of muscle were measured with bioimpedance (Fig. 3.) using an ImpediMed SFB7 bioimpedance spectrometer. Meat samples were measured at ambient abattoir temperature (~10° C) while held in a specially constructed jig. This jig maintained electrode spacing at 1 cm intervals. The SFB7 uses a tetrapolar electrode arrangement with two sense electrodes and two distally located current drive electrodes. Depending upon sample size, current drive electrodes were 20 cm apart (LL) or 6 cm apart (SM) with sense electrodes initially spaced 2 cm apart. Electrodes were stainless steel hypodermic needles (20g) penetrating 5 mm into the meat. Measurements were made in triplicate and repeated with sense electrode separation increasing in 2 cm steps, i.e. 2, 4, 6 cm etc to a maximum of 16 cm. Data were transferred to a PC and analysed according to the Cole model using manufacturer's software Bioimp v 4.17.0.0.

Fig. 3. Bioimpedance measurement of topside (SM – picture left) and loin (LL- picture right) muscle samples post mortem

3.4 Synchrotron measurements

Four separate synchrotron samples (A-D), 1 cm x 1 cm blocks were taken on the day of sampling (18 hours PM). For the Synchrotron SAXS beamline testing, fresh (36 hours PM) samples of 1 cm by 1 cm squares of around 2 mm thick were placed over holes in a metal plate and held in place with polyimide (Kapton) tape covering both sides to keep samples from drying out. All samples were mounted with muscle fibres running vertically as shown in Fig. 4.

Fig. 4. Four subsamples cut into 2 mm thin slices and fixed to the sample plate using tape for the SAXS testing.

Samples were analysed using the Australian Synchrotron SAXS/WAXS beamline (Kirby et al., 2013) using a Pilatus2-1M detector with approximately 2-5 x 10^{12} photons/s monochromatic 8.200 and 20.000 keV x-rays and a camera length of 7.33 m to cover q-ranges of 0.0015 – 0.1 $Å^{-1}$ and 0.003 to 0.3 $Å^{-1}$ respectively. Silver behenate was used to calibrate the q-scale of the instrument, and glassy carbon was used to calibrate scattering intensities per mm of sample thickness. Since the beam size (250 x 130 µm horizontal x vertical, full width at half maximum) was too small to provide a representative analysis volume in a single exposure, on-the-fly scans (i.e. frames acquired with the sample continuously moving), 110 two second exposures in a orthogonal grid (5 x 5 mm area of sample) were taken for each sub-sample (A, B, C or D). The SAXS image analysis was carried out using the program Scatterbrain. Whilst individual frames can be analysed (e.g. to spatially map structural features), frames from the whole grid were averaged in order to maximise the volume of analysis for each sub-sample. Measurements were also performed on Kapton tape for background subtraction.

Data was taken at 20 keV primarily to determine relative fat content from the intensity of the isotropic diffraction ring at 0.145 $Å^{-1}$. The absolute intensity scaling procedure accounts for total transmitted flux and scales to an absolute intensity (cm^{-1}) using an empirical scattering intensity sample (glassy carbon), but does not normalise for sample thickness, which had to be determined separately. This was done by empirically measuring the x-ray transmission of lamb meat samples at 20 keV of various thicknesses from 2-12 mm measured with a vernier gauge to determine the linear attenuation coefficient from the Beer-Lambert Law ($T = e^{-\mu t}$) where
(T = transmission, t = thickness, μ = linear attenuation coefficient).

Transmission = (I transmsitted sample / I incident sample) / (I transmsitted Kapton / I incident Kapton).

Relative transmitted flux and/or absolute transmission is easily measured in real time for every exposure on the beamline as transmitted flux is accurately measured by a detector inside the beamstop and incident flux is determined using an upstream foil-scattering detector.

The observed linear attenuation coefficient (0.7 cm^{-1}) was assumed to be constant with meat composition. The thickness of unknown specimens was thus determined as
T = - ln (t) / 0.07 (cm)

Absolute intensity corrected data was simply normalised by dividing by the sample thickness determined by transmission.

Two dimensional data was radially integrated into a one dimensional form where appropriate and the two major equatorial peaks used to determine the SAXS d-spacing of the actin/myosin fibril spacing (SAXS1 & SAXS2) and the cross sectional area of the hexagonal unit cell (Cell area). This two dimensional data was also used to determine a second peak representing the diffractions caused by fat content had the area under the curve calculated to determine Fat area, along with a calculation to determine Fat volume. The diagram below shows the fractions detected by SAXS beamline (samples collected during autumn) for myofibrillar proteins as denoted by ring A indicating actin and myosin peaks and muscle IMF as denoted by ring B indicating relative muscle fat area, respectively (Fig. 5). Fat area and Fat volume were measured by a computer program.

Peaks indicated by ‘A’ below in Fig. 5 explained: peak closest to centre is the lateral spacing between nearest myosin in angstroms while the peak further out from centre is the lateral distance of myosin to actin, or actin to actin in angstroms. The image at the top was taken at the 8.2 keV wavelength and the distance between the rings shown by A matched previous literature describing the distance between actin and myosin fibrils.

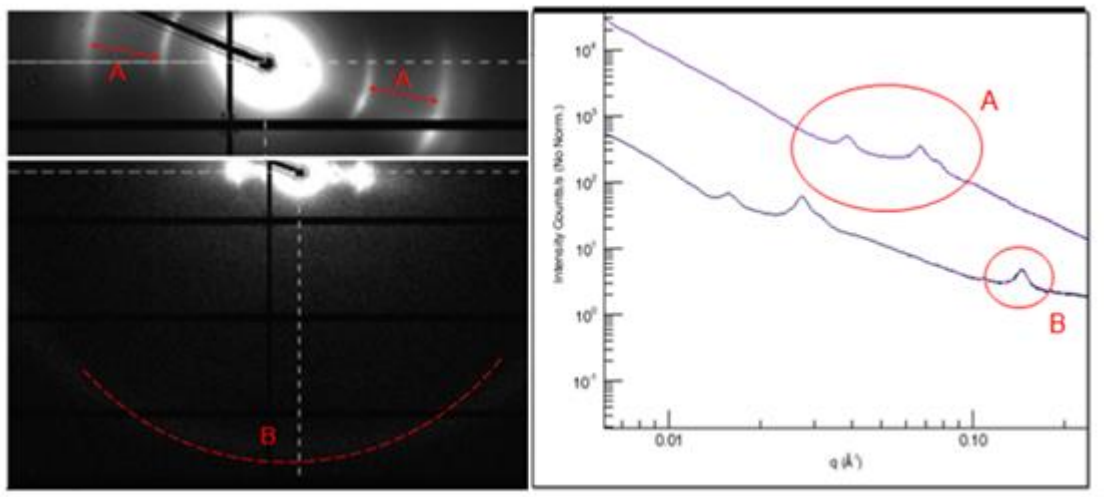

Fig. 5*. Preliminary results from SAXS beamline indicated that the oscillations shown above as A are the distances between Actin and Myosin filaments (measured at 8.2 keV) and the ring at B represents relative intramuscular fat (measured at 20 keV) with the graph showing the points where peak fitting and areas measures were carried out.*

3.5 Meat tenderness evaluation

The shear force samples were then taken, with 1 and 5 day samples alternated between caudal and cranial ends of the LL muscle. After the samples were aged for their allocated time periods (in vacuum packed bags at 4°C) they were then cooked. The fresh 65g samples were cooked for 35 min in plastic bags at 71°C in a waterbath. These were then left to cool overnight and then each block was sliced into six sub-samples with a cross-sectional area of 1 cm^2 and cut parallel to the muscle fibres. These subsamples were then used to measure peak force in newton (N) using a Lloyd (Model LRX, Lloyd Instruments, Hampshire, UK) with a Warner–Bratzler shear blade as described by Hopkins et al. (2011).

3.6 Intramuscular fat determination

Fat was extracted using a modified version of AOAC Method 960.39 (AOAC, 2007) for ether-extractable fat. A 40 g of wet muscle was completely freeze dried using a Dynavac, model FD 600 freeze dryer and ground into a fine powder using a FOSS Knifetech™ 1095 sample mill (FOSS Pacific, Unit 2, 112‑118 Talavera Road, North Ryde, NSW 2113). Approximately 1.5 g of ground sample was weighed in an extraction thimble and intramuscular fat extracted using petroleum spirit in a 250 ml round bottom glass flask set to soxhlet apparatus as described by Savell et al., (1986). Upon extraction for 7 hours at approximately 65ºC, thimbles were left to dry in the fume cupboard. IMF is calculated by weight difference of sample and adjusted to wet weight by using the individual freeze dried weights.

3.7 Collagen determination

Collagen samples were taken and placed into sample tubes and frozen 1 day PM. 25 g samples of muscle tissue were then weighed out into flat bottomed 50ml tubes and freeze dried. These were then ground to produce a fine powder and used to determine total collagen and soluble collagen. To determine total collagen, 0.10 g of meat powder was weighed into hydrolysis tubes (in triplicate) with 3 ml H_2SO_4 (3.5 M) added. The tubes were then covered with marble and heated for 16 h (overnight) at 105°C. The hot hydrolysate was then transferred into a 50 ml tube with the aid of water and added up to 50 ml with water.

This is then capped and shaken well before part of the solution was filtered (using glass wool in 10 ml pipet tips) into a 15 ml Falcon tune (filtrate is stable for at least 2 weeks at 4°C). 1 ml of the filtrate is diluted with 3.75 ml water and 0.25 ml 2 M NaOH and 0.5 ml of the diluted filtrate is pippeted into a test tube (in triplicate). 0.25 ml of oxidant solution is added to each tube, vortexed, and let stand for 20 min at room temperature. 0.25 ml of colour reagent was added to each tube, vortexed, and covered with foil, then placed in a 60°C waterbath for 15 min. The tubes were then cooled under running tap water for at least 3 min and the absorbance of the solution measured at 558nm. To determine soluble collagen, 1.5 g of meat powder was weighed into a 50 ml tube. This then had 10 ml of water added, vortexed, and heated for 2 h in a waterbath of 80°C, with mixing every 1/2 h. Insoluble material is the pelleted by spinning for 30 min. centrifuge at maximum speed and the supernatant is filtered through a Whatman no. 1 filter. 0.5 ml of the supernatant is transferred into a hydrolysis tube (in duplicate) with 3 ml H_2SO_4 (3.5 M) added, covered with marble, and heated for 16 h (overnight) at 105°C. The hot hydrolysate was then transferred into 15 ml Falcon tubes with the aid of water and with up to 10 ml of water added (filtrate is stable for at least 2 weeks at 4°C). 1 ml of filtrate was diluted with 1 ml 1.0 M NaOH, then 0.5 ml of the pH adjusted sample was pippeted into a test tube (in triplicate). 0.25 ml of oxidant solution was added to each tube, vortexed, and let stand for 20 min at room temperature. 0.25 ml of colour reagent was then added each tube, vortexed, and covered with foil. These tubes were then placed in a 60°C waterbath for 15 min, the cooled by running tap water over them for at least 3 min. Absorbance of the solution was then measured at 558nm. To determine the final collagen content a calibration curve of 0, 0.6, 1.2, 1.8 and 2.4 µg hyroxyproline per ml H_2O assayed for hydroxyproline content (in duplicate) as described above (0.5 ml sample, 0.25 ml oxidant solution, 0.25 ml colour reagent etc.) was used (AOAC official method 990.26 (AOAC Official Methods of Analysis, 2000).

3.8 Determination of sarcomere length and particle size

The sarcomere length (SL) and particle size (PS) samples were removed from the side of the loin muscle (where the muscle fibres run diagonally along the surface), wrapped in foil and placed in snap lock bags. The SL and one of the PS samples were aged for 1 day post slaughter before being frozen (-20°C), while the remaining PS sample was aged for 5 days at 4° C before being frozen. The method for the determination of sarcomere length (SL) was similar to that previously described by Bouton et al. (1973). For PS, one gram samples were prepared by slicing along the fibre direction, avoiding any visible fat or connective tissue from the frozen muscle samples. Further details of the method are given by Karumendu et al. (2009).

3.9 Statistical Analyses

3.9.1 Bioimpedance spectroscopy

Data were obtainable for 49/50 loin samples (lambs and sheep) and 48/50 topside samples (lambs and sheep). Of these, useable data were available for 47 loin samples and all topside samples. Lost data was due to high frequency noise in the data for these two samples. This was most likely due to stray capacitance while recording measurements. This is a well-recognised problem in BIS measurements. Data were obtainable for all 50 loin samples (lambs and sheep) and all topside samples (lambs and sheep).

Bioimpedance analysis (BIA) is the measurement of the response of biological tissues to the application of an external electric current. Bioelectrical impedance techniques measure the opposition of the body's tissues to the flow of an electrical current. This opposition is known as impedance where the applied electrical current is an alternating current and is, by

convention denoted by the symbol, Z. Impedance comprises two components, reactance (Xc) and resistance (R) (Fig. 6).

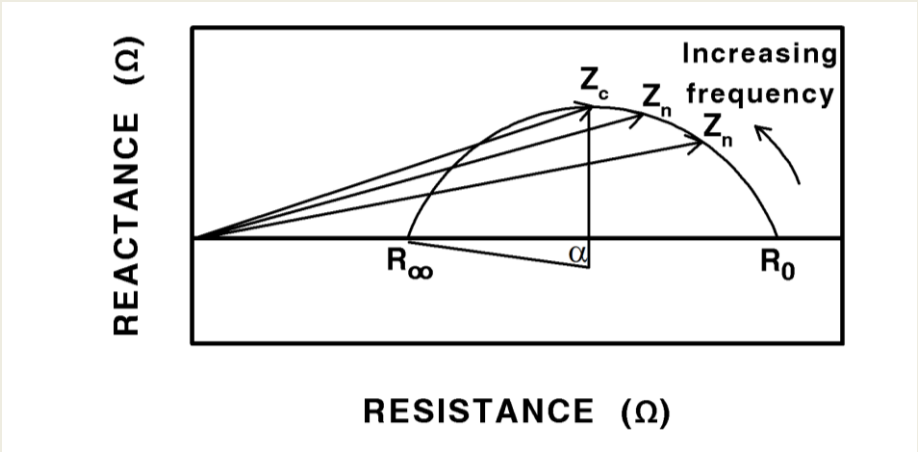

Fig. 6. *Relationship between impedance (Z), resistance (R) and reactance (Xc). Z_n represents impedance measured at a specific frequency of the applied electric current. Z, R and Xc, measured over a range of frequencies, when plotted as Xc versus R describe a semi-circle known as a Cole plot. The centre of the plot is depressed below the resistance axis and subtends an angle (α) with R∞.*

Reactance is the opposition of current flow due to the cell membranes and tissue interfaces whereas resistance is the opposition to the flow of electric current through tissue fluids. The electrical model describing biological tissue is that of Cole (Fig. 7).

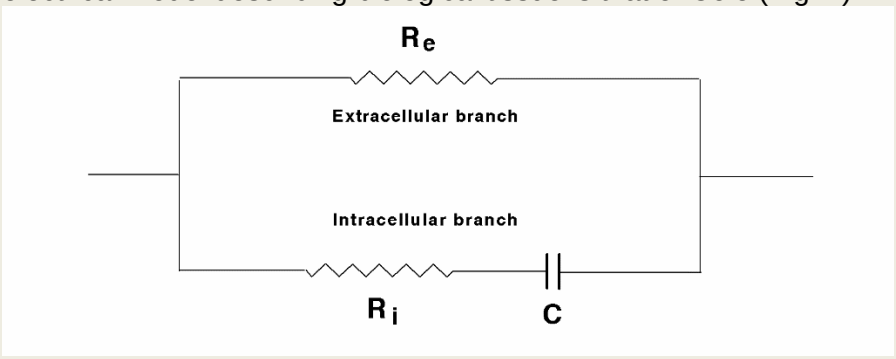

Fig. 7. *Electrical model describing the electrical properties of biological (cellular) tissues.*

This model envisages that biological tissue as a matrix of cell, each surrounded by a membrane that exhibits electrical capacitance (C) enclosing intracellular fluid that has a an electrical resistance (R_i) with cells surrounded by extracellular fluid which also has resistance (R_0 or R_e). Tissue is a matrix of many individual Cole circuits. The magnitude of resistance is inversely proportional to the volume of the conductor and is determined by the inherent electrical properties of the conductor, the specific resistance or resistivity (ρ). The magnitude of resistance is also proportional to conductor length. These relationships are described by

$$V = \rho \frac{l^2}{R} \qquad \text{Equation 1}$$

where V is volume (mL), ρ is resistivity (ohm.cm), l is conductor length (cm) and R is resistance (ohm).

In BIA the applied current is an alternating current, i.e. with a sine wave of a given frequency, typically 50 kHz. In bioimpedance spectroscopy (BIS) alternating currents are applied over a range of frequencies, typically between 5 and 1000 kHz. At low frequencies, the capacitive nature of cell membranes is such that present a barrier to current flow and current flows only through the extracellular fluids while at high frequencies current can pass across the cell membranes and current flows through both intra- and extracellular water, i.e. total tissue water (Fig. 8).

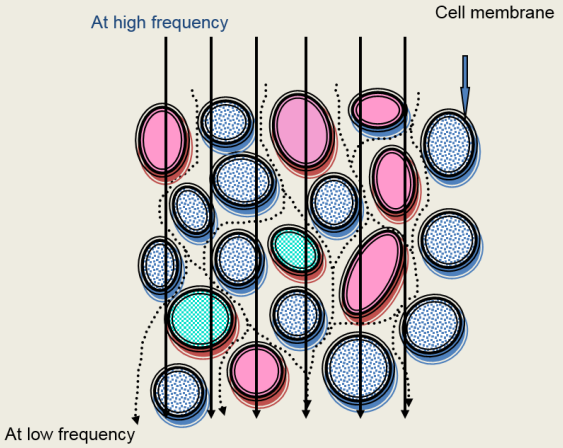

Fig 8. *Current flow through biological tissues.*

In practice, when BIS is applied to tissues, Z, R and Xc at each applied frequency of current is obtained. These data may be depicted graphically in the form shown in Fig 1. A number of parameters defining this plot can be determined:

R_0 resistance at zero frequency, i.e. that of the extracellular fluid
$R\infty$ resistance at infinite frequency, i.e. that of total tissue fluid
R_i resistance of intracellular fluid, computed from R_0 and R_∞
Zc impedance at maximal reactance and at the characteristic frequency (fc) for this tissue composition
Cm cell membrane capacitance
α alpha, the angle defining the depression of the plot centre due to distributed time constants.

Additional information may also be determined. In equation 1 if R is the measured resistance at a low frequency, ideally zero (R_0), then the volume of the extracellular fluid may be calculated if the resistivity of extracellular fluid and the conductive length is known. Equally, if R_∞ or R_i is known then total tissue fluid and intracellular fluid volumes may be calculated. Furthermore, if the hydration of tissue is known, then total tissue water can be converted to an estimate of fat-free mass on the basis that it is this tissue compartment that is electrical conductive. This is the basis of the use of impedance technologies for body composition analysis.

3.9.2 Synchrotron SAXS technology

The statistical analysis was undertaken by fitting mixed models using Asreml software package (Butler, 2009) and was run under R (R Core team, 2013). The results for Shear force samples aged 1 day (SF1), shear force samples aged 5 day (SF5) and intramuscular fat (IMF) on each of the carcases sampled were regressed on a number of traits associated with each carcase, either excluding or including synchrotron traits which were averaged over

the four slices (A-D). Each mixed model consisted of a random term for lot. Traits not significant at the 5% level were removed in turn via backwards stepwise regression.

The following initial models were used (excluding the synchrotron traits):

SF1 ~ Type + Pos + Kill + SDate + pHu + AdjSL + AdjPS1 + Tot Coll + Sol Coll + *Lot* + *error*

SF5 ~ Type + PosnSF5 + Kill + SDate + pHu + AdjSL + AdjPSA5 + TotColl + SolColl + *Lot* + *error*

IMF ~ Type + SDate + GR + Tot Coll + Sol Coll + *Lot* + *error*

In these models the notation terms are Type (Sheep or Lambs), Pos (cranial or caudal), Kill (Autumn or Spring), SDate (Day of slaughter), pHu (ultimate pH), AdjSL (Mean adjusted for missing values), AdjPS1 and AdjPS5 (mean adjusted for missing values, 1 or 5 days aged respectively), Tot Coll (total collagen content) and Sol Coll (concentration of soluble collagen).

A second set of models were also considered, but with the addition of the synchrotron traits SAX1+SAX2+Cell area as well. Either trellis plots or added variables plots were used to display the relations between the response and the retained variables (as well as highlight any issues with the regressions, such as outliers). Added variables plots for mixed models were constructed as per the method of section 3.1.1 of Hilden-Minton (1995).

4 Results

4.1 Bioimpedance spectroscopy

4.1.1 Loin meat sample characteristics

LL samples were all cut similarly and were of rectangular shape and of similar weight, 354.8 ± 52.2 g wet weight (autumn) and 408.6 ± 66.4 g wet weight (spring). BIS measurements were performed using the central section of the cut centred at the mid-point along the muscle fibre axis. At the mid-point LL widths were 63.9 ± 6.2 mm and 24.7 ± 3.3 mm (autumn) and 65.6 ± 5.5 mm and 23.5 ± 3.2 mm (spring) and were of relatively even thickness throughout the sample. Furthermore, fibre orientation was consistently (by visual inspection) longitudinal. This is important when attempting to relate impedance data to the conductive characteristics of the tissue not confounded by geometrical constraints or anisotropy. Estimated BIS-measured sample weights (measured tissue volumes) (at 16 cm electrode spacing) were 197.4 ± 29.0 g (autumn) and 225.6 ± 38.7 g. These data are calculated assuming that current flow is homogeneous across the complete cross-sectional area of the meat cut and that measurement is of the total volume between the electrodes.

Impedance spectra

Typical spectra at 6-cm inter-electrode spacing for a loin sample are shown in Fig. 9 and are typical of a Cole model electrical system. Generally data were well fitted to the model semi-circular plot (centre panel). Triplicate measurements (at 1 s intervals with a measurement period of 1 s) were all virtually identical.

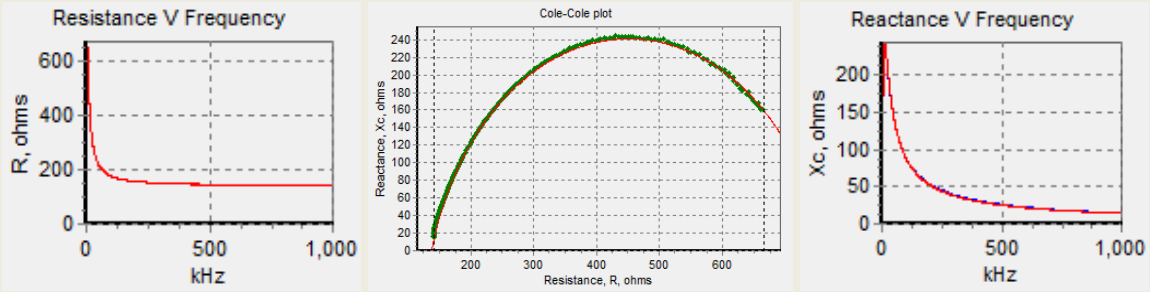

Fig. 9. *Impedance spectra for loin sample.*

Resistance (e.g. at zero frequency, Fig. 10) increased linearly with inter-electrode distance as expected according electrical theory and to equation 1. Since resistance varies linearly with distance for a body of constant cross-section but inversely with cross-sectional area, these data are consistent with the samples being of relatively uniform shape as indicated by the geometric measurements.

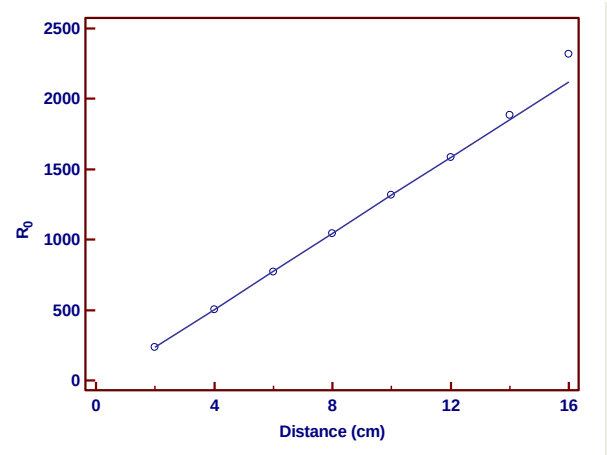

Fig. 10. *Relationship of measured resistance with inter-electrode distance*

Similar plots were observed for R_i, Zc and R_∞.

Spectral characteristics – fc

The mean characteristic frequency, fc, was 12.7 ± 4.1 kHz for lamb samples and 11.6 ± 3.4 kHz for sheep samples at 16 cm spacing; 12.8 ± 5.4 kHz for lamb samples and 12.0 ± 5.1 kHz for sheep samples at 2 cm spacing. Lack of fc varying with inter-electrode distance indicated homogeneity of the tissue throughout the sample. There was a tendency, although not significant (two-way ANOVA), for fc to be slightly higher in spring animals compared to autumn animals (Fig. 11).

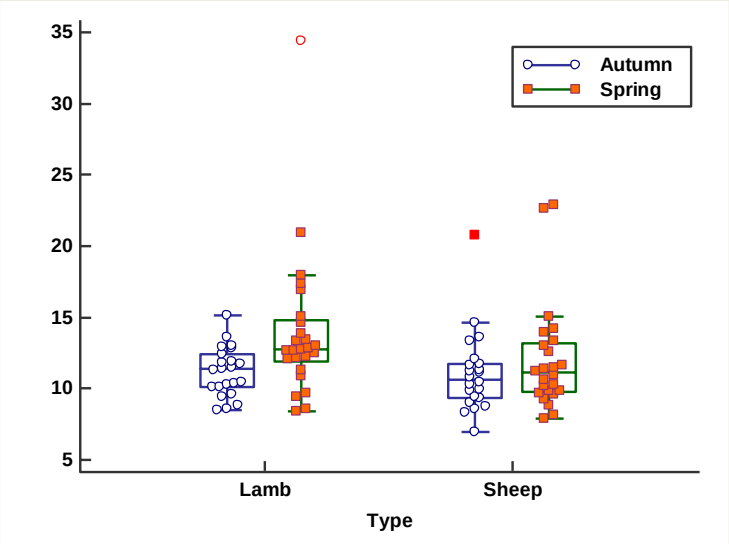

Fig. 11*. Distribution of fc values by season and animal type.*

Spectral characteristics – Cm

The mean membrane capacitance, Cm, was 6.1 ± 0.96 nF for lamb samples and 5.9 ± 1.1 nF for sheep samples at 16 cm electrode spacing. There was no significant effect of season (Fig. 12).

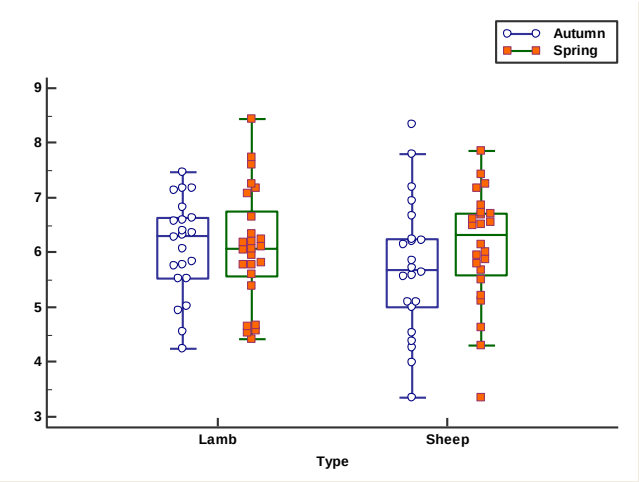

Fig. 12*. Distribution of Cm values by season and animal type.*

Membrane capacitance varied with inter-electrode distance, declining exponentially as distance increased. These data are consistent with the concept that the meat sample is an array of Cole circuits; as the separation of the sense electrodes increases, more Cm elements are in series, and the result of adding capacitors in series is to decrease the overall capacitance. This is exactly analogous to increasing R_0 and R_∞ as the separation increases. As can be seen from Fig. 13 below there appears to be no difference in the pattern between lamb and sheep samples. There was a weak (r = 0.3) but almost significant (*P* = 0.053) relationship between Cm and shear force. This observation although preliminary is encouraging since shear force is possibly the meat characteristic most closely related to meat quality in terms of tenderness.

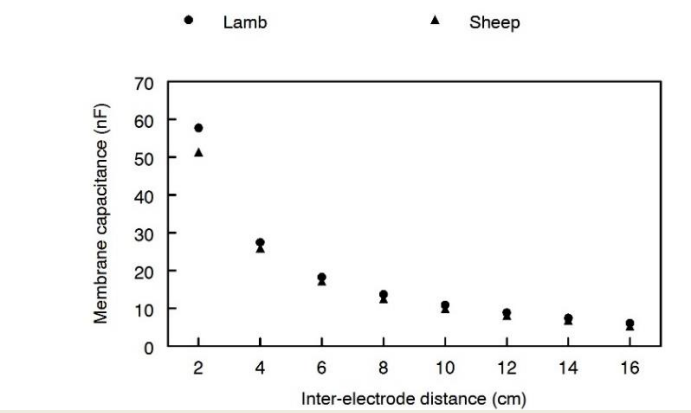

Fig. 13*. Mean change in membrane capacitance with inter-electrode distance for sheep and lamb loin samples.*

Spectral characteristics – alpha

The mean alpha angle, α, was 46.6 ± 3.9° for lamb samples and 47.9 ± 1.5° for sheep samples. α did not vary with inter-electrode distance again indicating homogeneity of the tissue throughout the sample. There was a slight but again non-significant tendency for there to be a seasonal effect upon both the impedance ratios and alpha (Figs. 14 & 15). This observation, along with the small seasonal effect on fc noted above, suggests that the Cole plots may be subtly different between samples obtained in the different seasons. This difference is not, however, captured by any single parameter that describes the shape of the Cole plot. It would be fruitful to assess differences between samples obtained in the different seasons using cluster analysis of encompassing multiple descriptors of the plots.

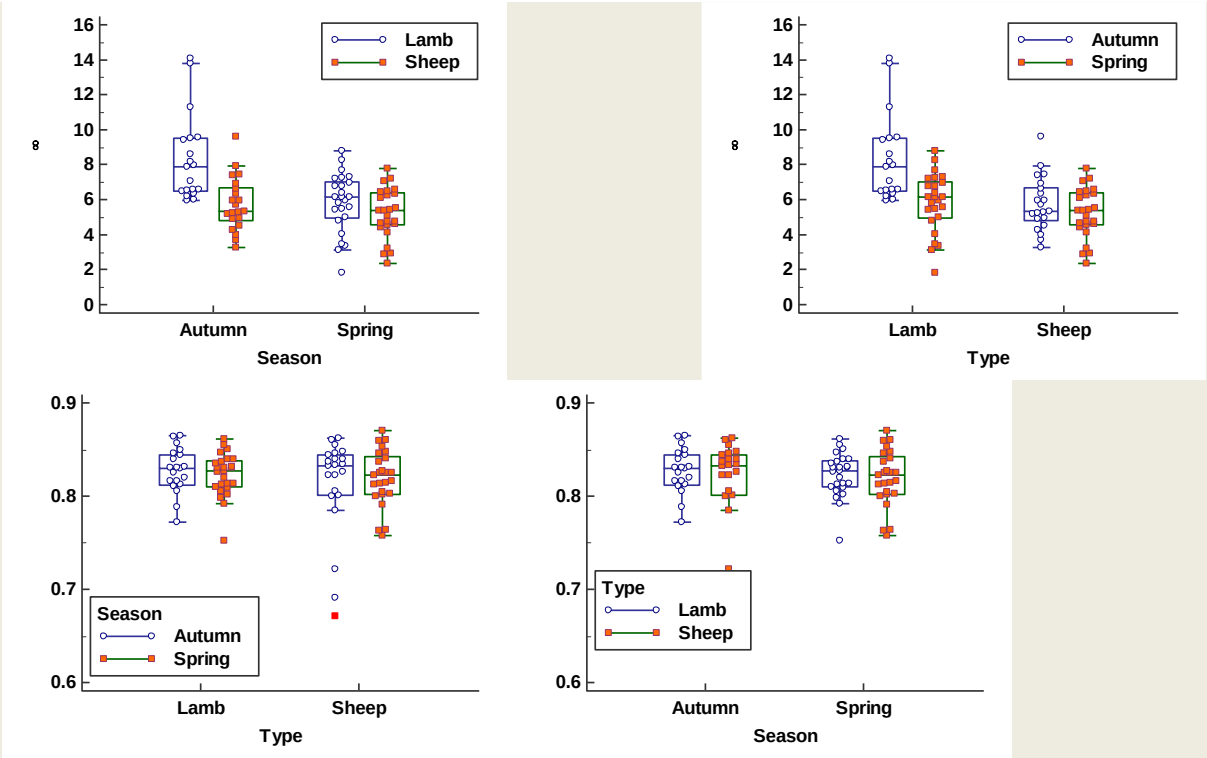

Fig. 14. *Ratio of R0 and R∞ values for loin samples at 2 cm electrode spacing (top panels) and alpha angle (radians) (bottom panels)*

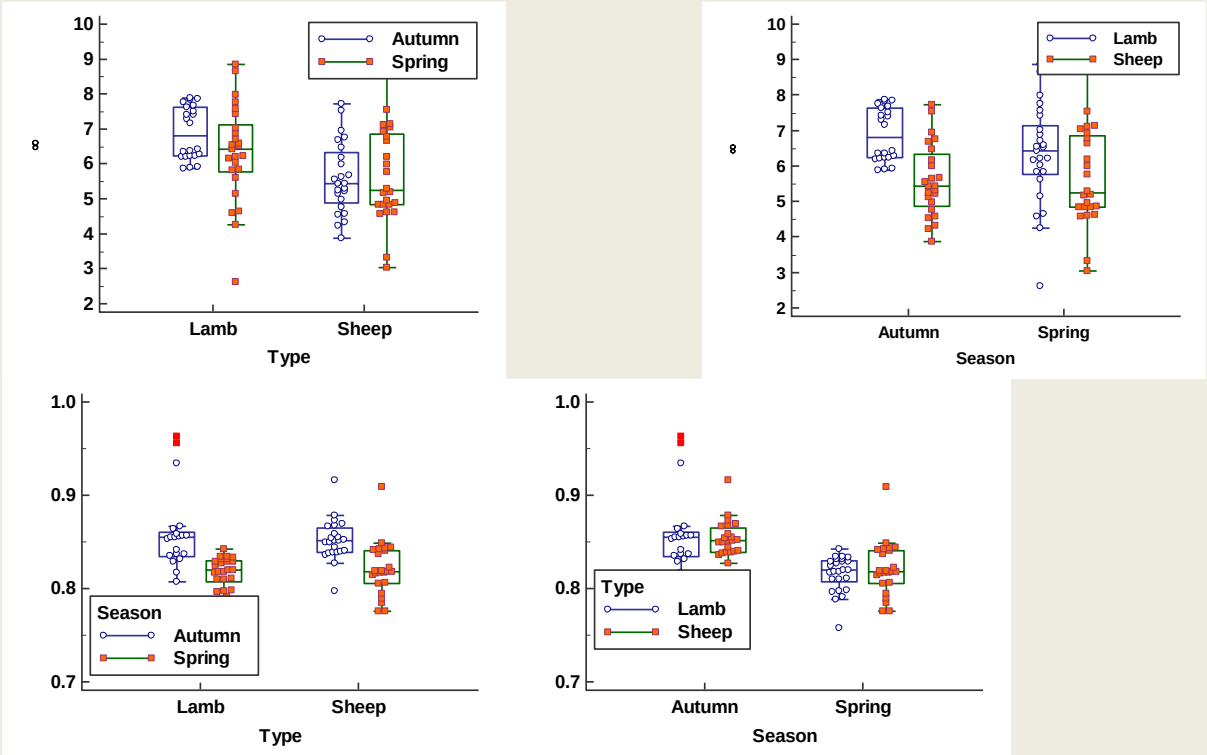

Fig. 15. *Ratio of R0 and R∞ values for loin samples at 16 cm electrode spacing (top panels) and alpha angle (radians) (bottom panels)*

Tissue resistivity

Resistivity can be calculated from the measured impedance and the sample volume and inter-electrode distance according to Equation 1. This equation assumes, however, a simple homogeneous cylindrical sample geometry. This is not the case for the meat samples used here and Equation 1 may be modified according to Hanai mixture theory by incorporating a shape factor Kb (Jaffrin et al. 2006). Figs. 16 & 17 show the resistivities calculated according to equation 1 ("split-plot") and the Hanai methods. Also shown (horizontal line) is a published values for bovine muscle (Geddes and Baker, 1967); data for sheep are lacking.

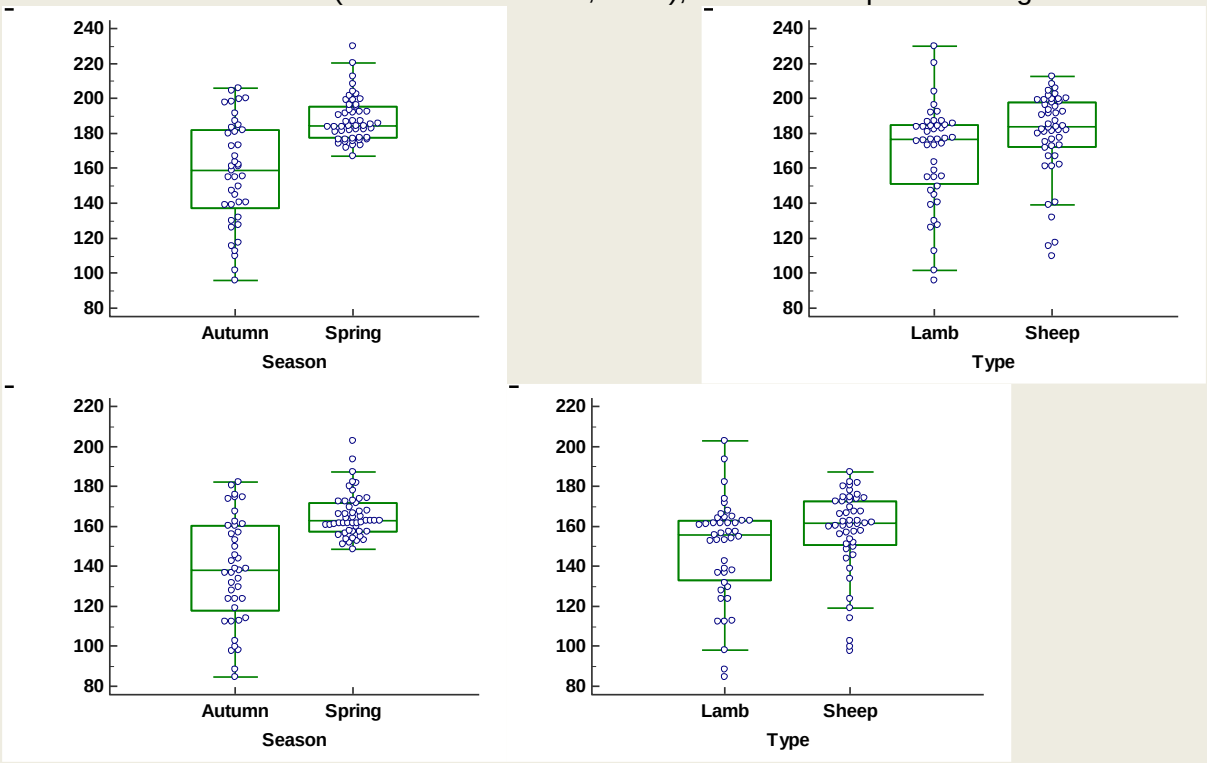

Fig. 16. *Specific resistivity of total tissue water based on equation 1 (upper panels) or Hanai theory (Lower panels) for 2 cm electrode spacing.*

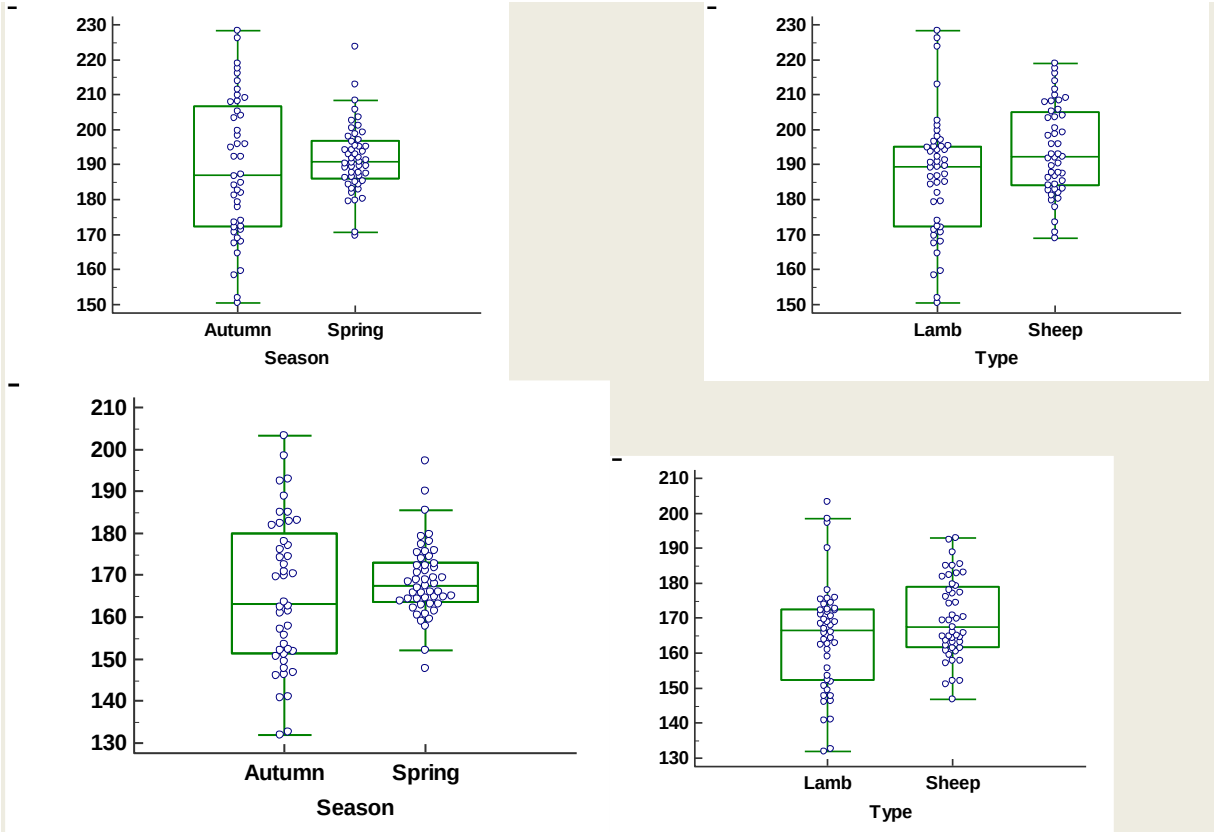

Fig. 17. *Specific resistivity of total tissue water based on equation 1 (upper panels) or Hanai theory (Lower panels) for 16 cm electrode spacing*

Prediction of fat-free mass

There were strong correlation (r = >0.7, P < 0.0001, Figs. 18 & 19) between predicted fat-free mass and that calculated from the chemically determined water content of meat samples, irrespective of method of calculation.

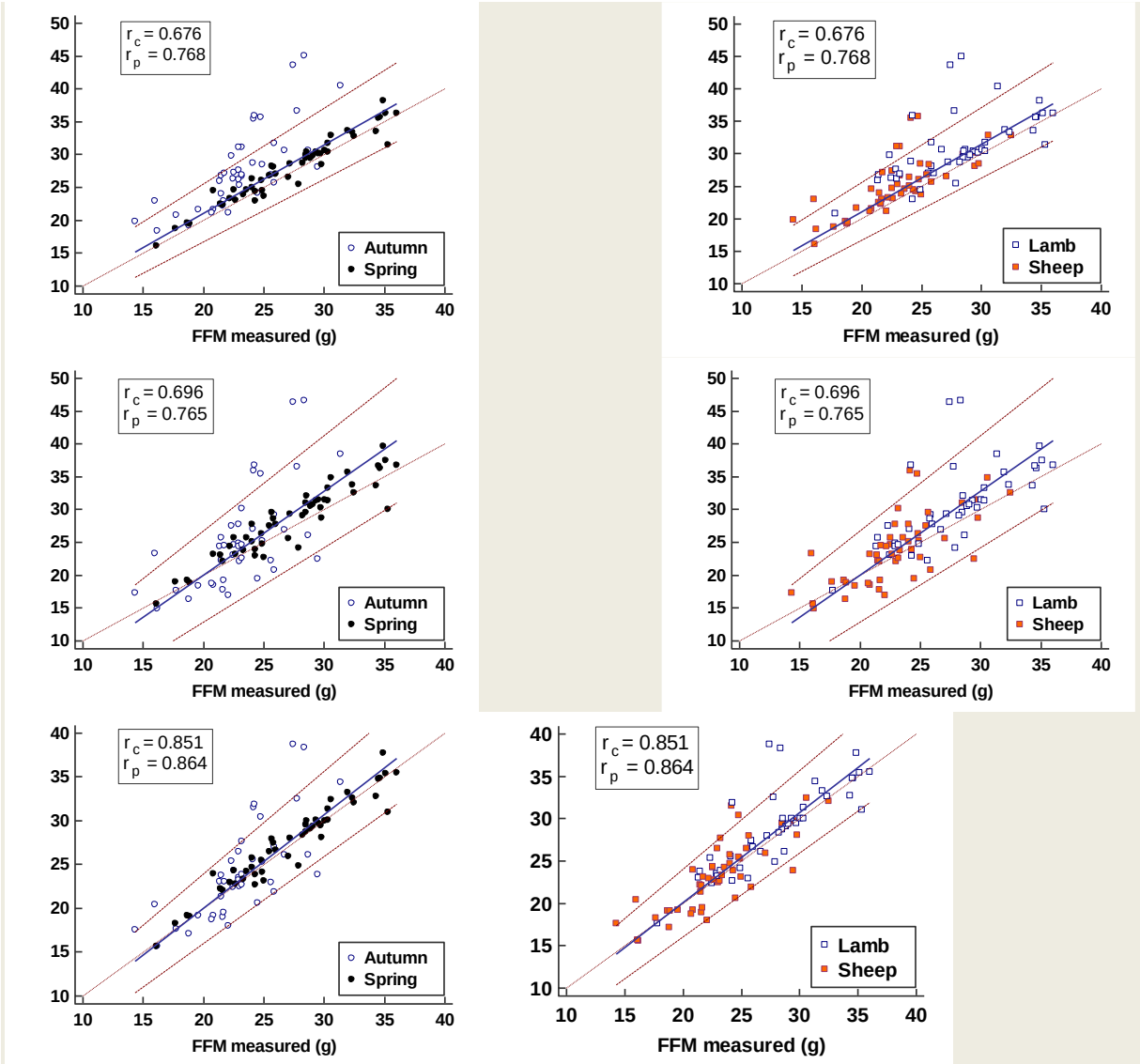

Fig. 18. *Relationship between predicted fat-free mas and fat-free mass determined chemically – 2 cm electrode spacing. Upper panels calculated according to Hanai mixture theory using a fixed resistivity of 170 ohm.cm (Geddes and Baker, 1967;* Jaffrin et al. 2006*), middle panels based on Equation 1; lower panels based on Hanai mixture theory using the mean resistivity determine for half of the sample (split-plot cross-over design).*

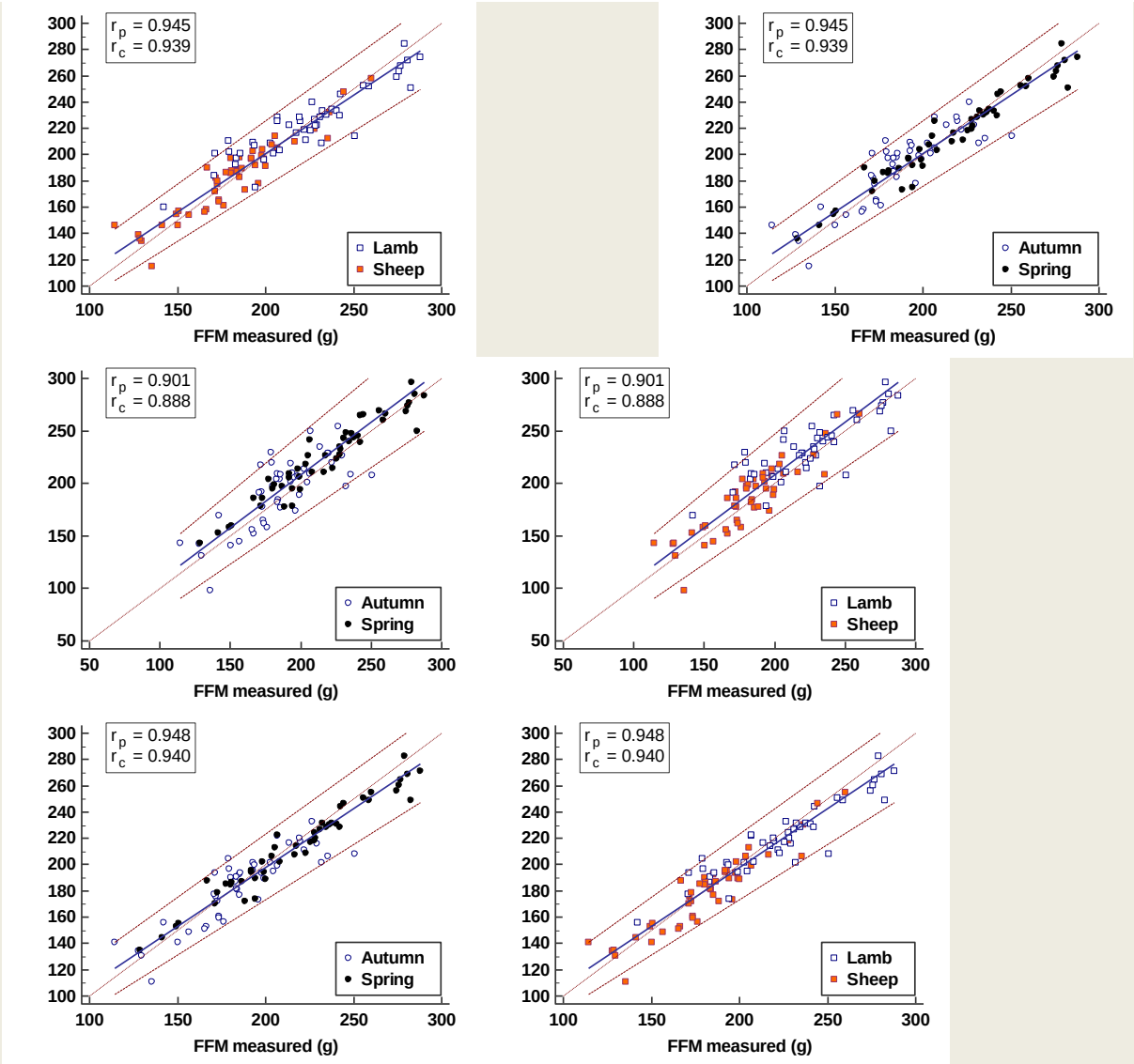

Fig. 19. *Relationship between predicted fat-free mas and fat-free mass determined chemically – 16 cm electrode spacing. Upper panels calculated according to Hanai mixture theory using a fixed resistivity of 170 ohm.cm (Geddes and Baker, 1967;* Jaffrin et al. 2006*), middle panels based on Equation 1; lower panels based on Hanai mixture theory using the mean resistivity determine for half of the sample (split-plot cross-over design).*

The highest correlations were observed for the 16 cm electrode spacing data. This is not entirely surprising since errors in measurement will be proportionally larger when making measurements of small sample volumes. Also, it is likely that in such small samples homogeneity of current flow cannot be guaranteed. Consequently, it is possible that the electrical sampled volume is not of the measured physical volume. Correlations of the magnitude observed in the 16 cm spacing samples are typical of those when BIS is used to predict fat-free mass in animals in vivo. Fat content of the meat samples was low, mean 9.4 g (4.9 ± 2.5% wet weight). Fat mass in BIS is determined by subtraction of the predicted FFM from total weight. Consequently, errors associated with the prediction of FFM (residual SD = 14.28 g, 5.3%) are magnified when used to predict fat content. Unfortunately this can lead to prediction of negative fat contents. BIS is not suitable to predict fat content when fat content is low (Figs. 20 & 21).

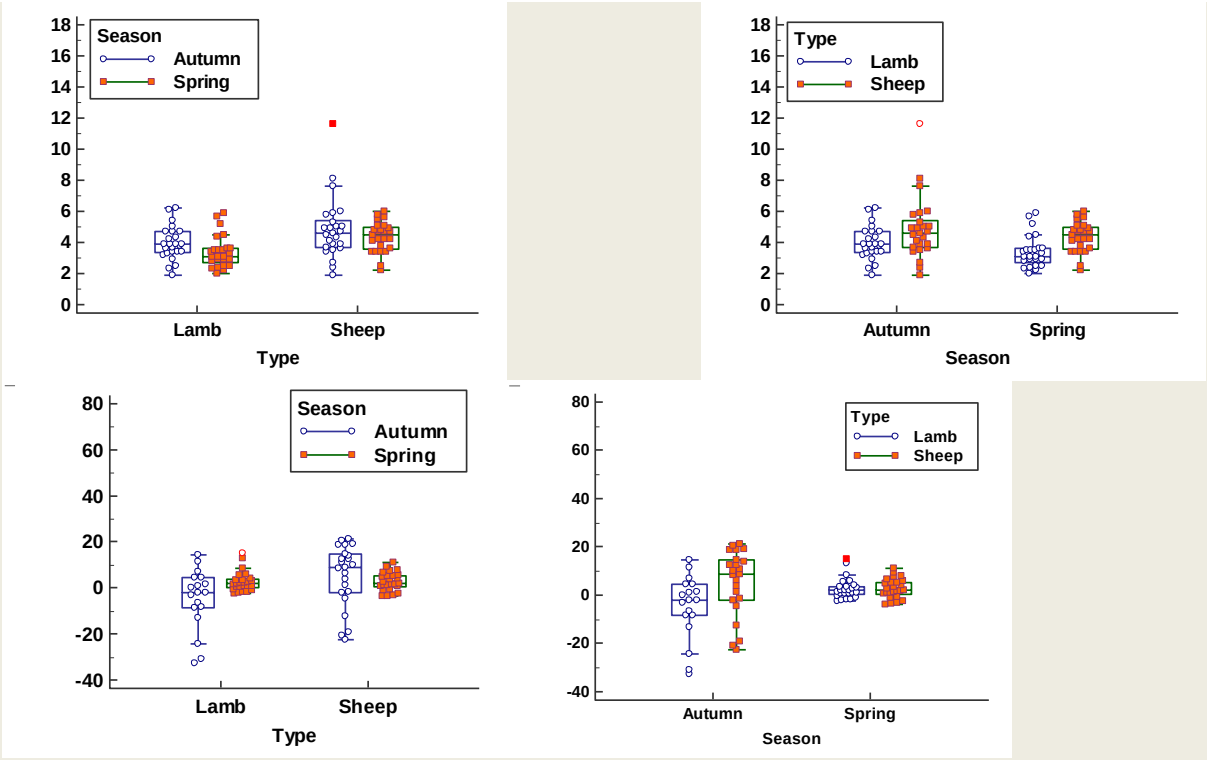

Fig. 20. *Relationship between predicted %fat mass (lower panel) and %fat mass determined chemically (upper panel) – 2 cm electrode spacing. Predicted fat based on split-plot prediction equations* (see Fig. 18).

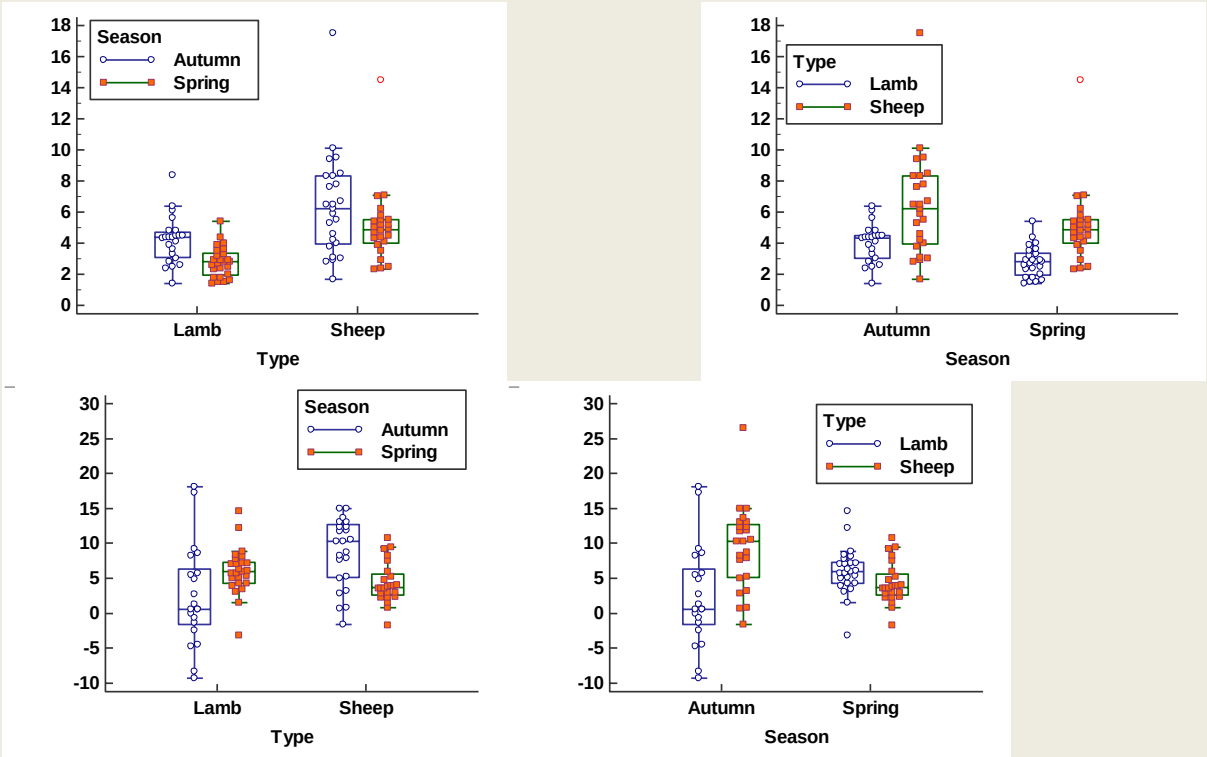

Fig. 21. *Relationship between predicted %fat mass (lower panel) and %fat mass determined chemically (upper panel) – 16 cm electrode spacing. Predicted fat based on split-plot prediction equations* (see Fig. 19).

4.1.2 Topside meat sample characteristics

Topside samples gave more variable results reflecting the less optimal geometry of the samples; more cuboidal than oblong. In addition, these samples showed marked anisotropy due to the mixed orientation of the muscle fibres. It, therefore, proved extremely difficult to obtain reproducible samples (Figs 22 – 24). Owing to anisotropy, resistivities were higher than those obtained for loin muscle samples.

Topside muscle resistivity (4 cm spacing)

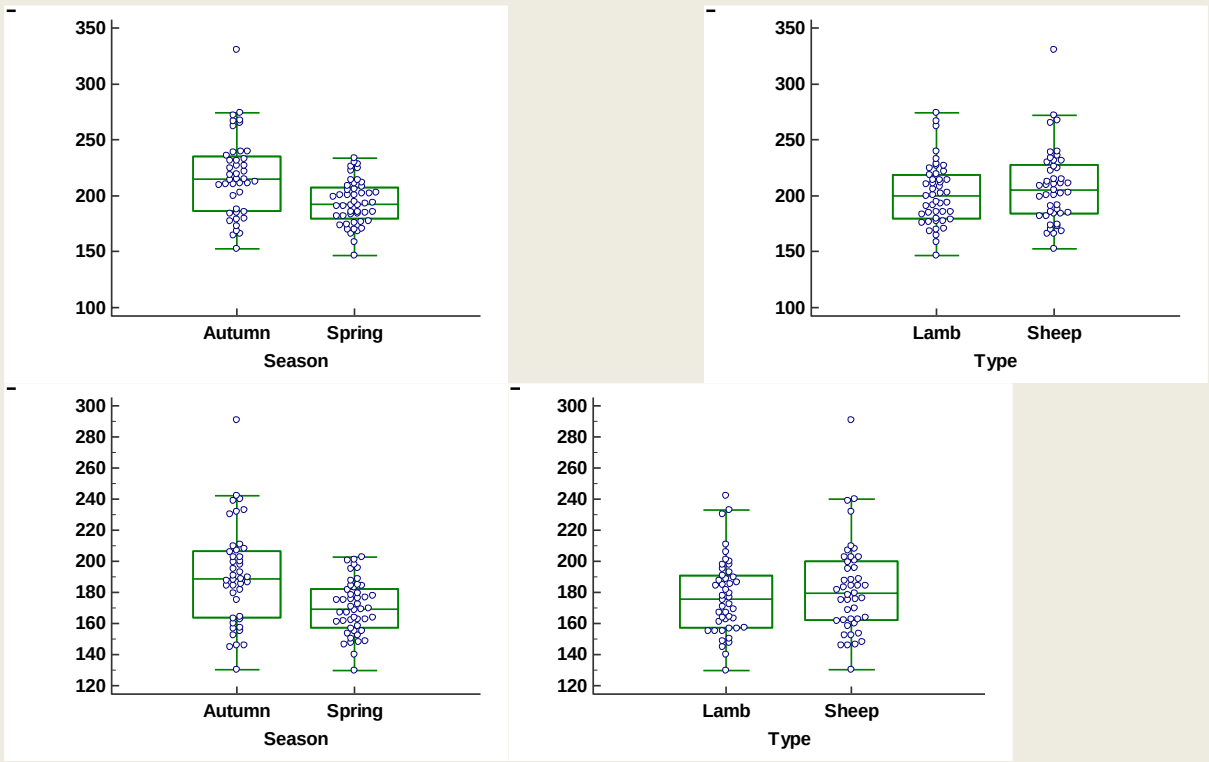

Fig. 22. *Specific resistivity of total tissue water in topside muscle based on equation 1 (upper panels) or Hanai theory (Lower panels) for 4 cm electrode spacing.*

Correlation of predicted fat-free mass with measured fat-free mass in topside muscle
Strong correlations were again observed between predicted fat-free masses and chemically-determined fat-free mass but larger variation in data were observed (Fig. 23).

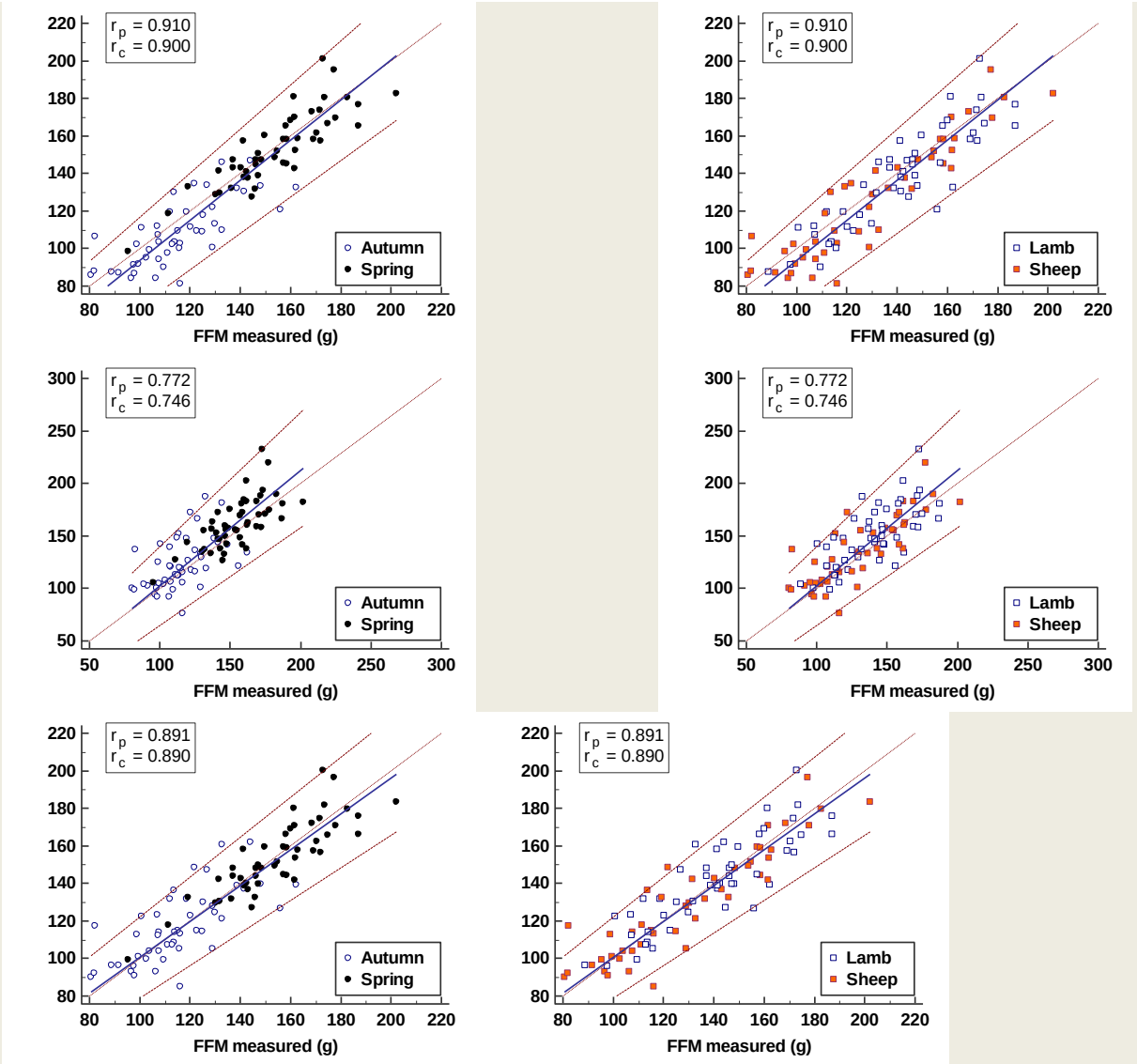

Fig. 23. *Relationship between predicted fat-free mass and fat-free mass determined chemically in topside muscle – 4 cm electrode spacing. Upper panels calculated according to Hanai mixture theory using a fixed resistivity of 170 ohm.cm (Geddes and Baker, 1967* Jaffrin et al. 2006*), middle panels based on Equation 1; lower panels based on Hanai mixture theory using the mean resistivity determine for half of the sample (split-plot cross-over design).*

Percent (%) fat mass determination in topside muscle

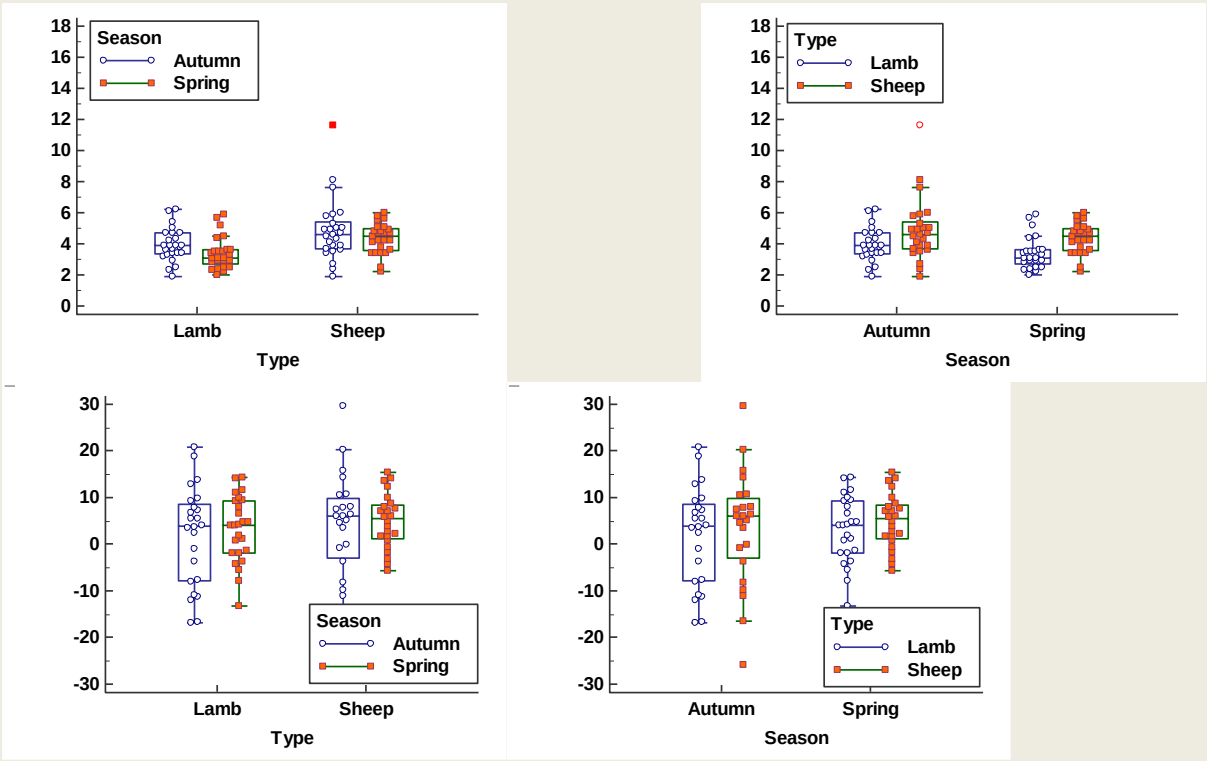

Fig. 24. *Relationship between predicted %fat mass (lower panel) and %fat mass determined chemically (upper panel) in topside muscle – 16 cm electrode spacing. Predicted fat based on split-plot prediction equations* (see Fig. 23).

4.2 Synchrotron SAXS technology

The variation in shear force 1 day (SF1), shear force 5 day (SF5) and intramuscular fat content (IMF) from both spring and autumn kills is shown in Fig. 25.

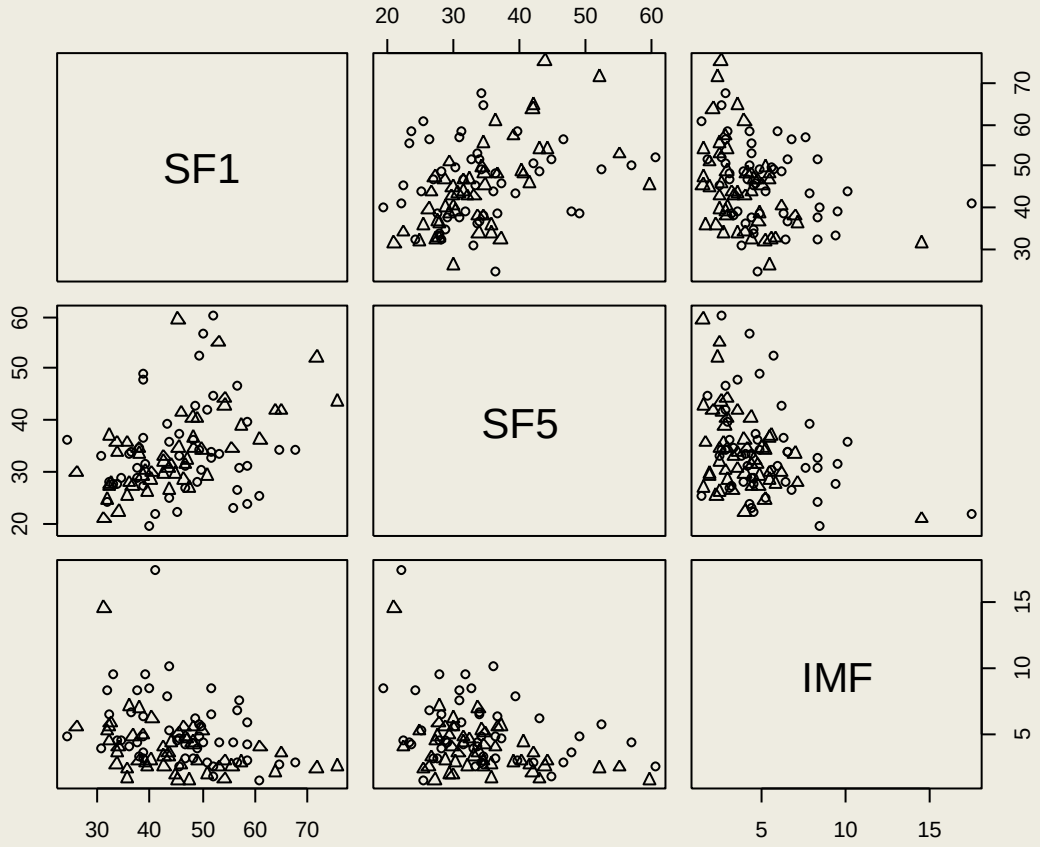

Fig. 25. Pairs plots of SF1, SF5 and IMF. Spring samples as triangles, Autumn as circles.

The variation within the SAXS variables (Cell area, Fat area, Fat volume [Fat vol], SAXS1, SAXS2) by the position within the LL (slice) and according to the season the animals were killed (Autumn or Spring) is shown in Fig. 26.

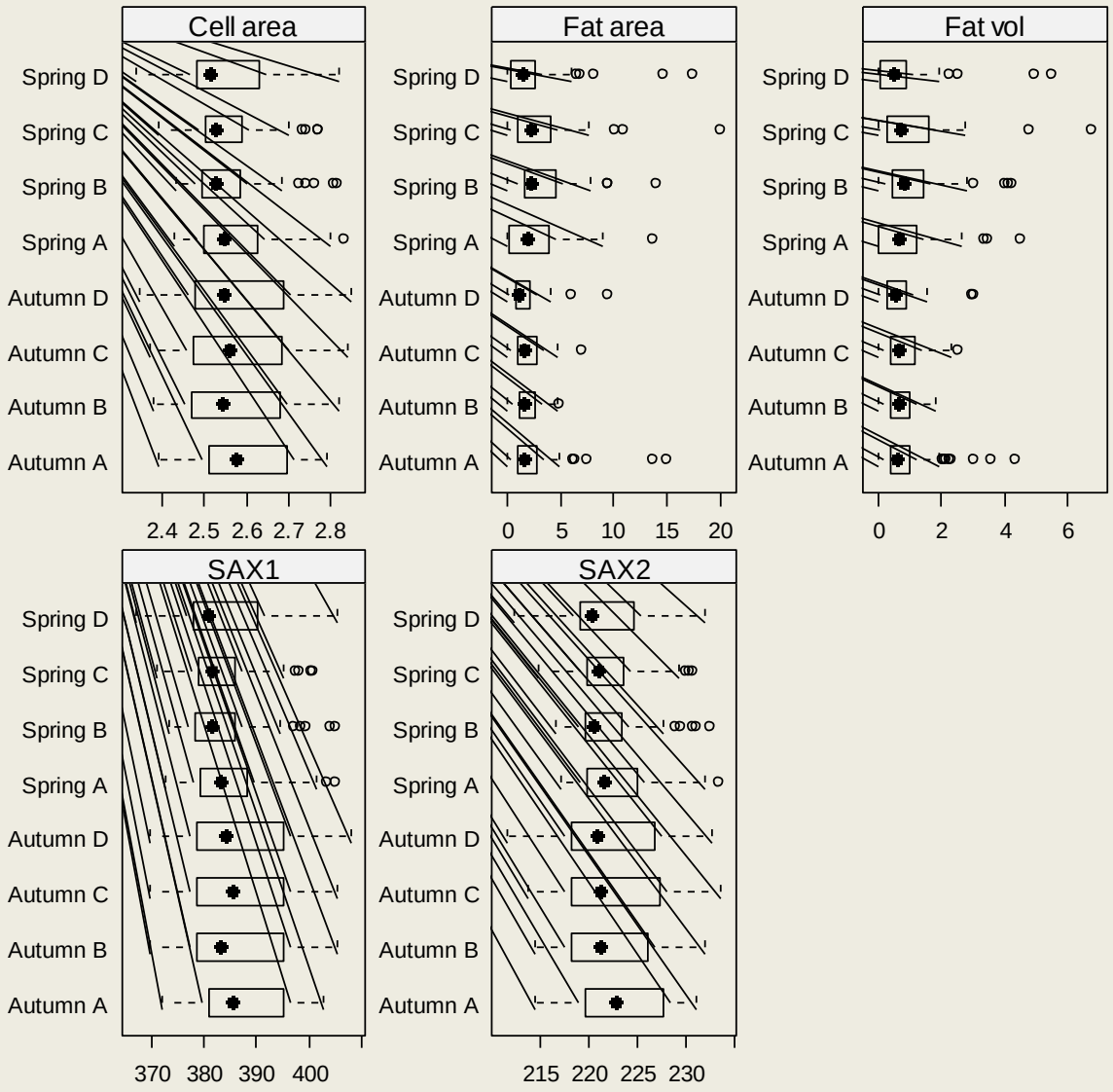

Fig. 26. Box plots of SAXS variables by sample position (slice A-D) and kill season (autumn, spring).

Initially, the SAXS results were not included within the models to determine the relationships between meat quality measures and shear force. Ultimate pH (pHu) and sample position in the LL (cranial or caudal) had a significant effect on shear force measured on 1 day (SF1) with $P < 0.03$ and $P < 0.01$, respectively (see Fig. 27).

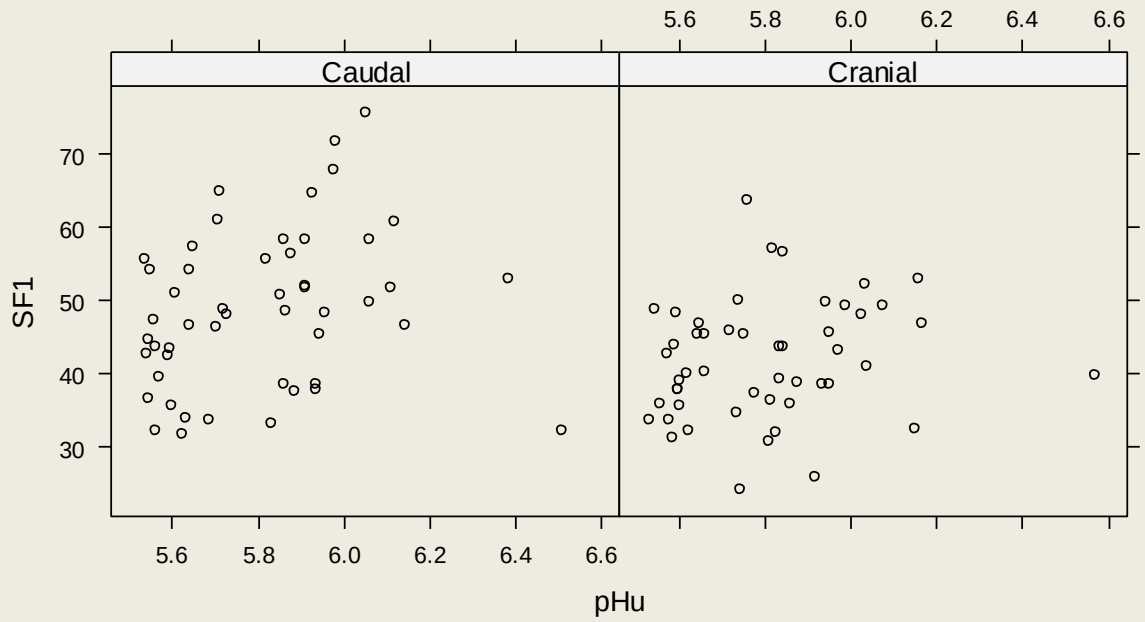

Fig. 27. Relationship between predicted shear force (SF1) and pHu according to position within the LL

Shear force of the LL 5 day post mortem was significantly influenced by the concentration of collagen ($P < 0.01$), along with an interaction between the type of animal (sheep or lamb) and position (cranial or caudal) of the sample in the LL ($P < 0.01$) (Fig. 28).

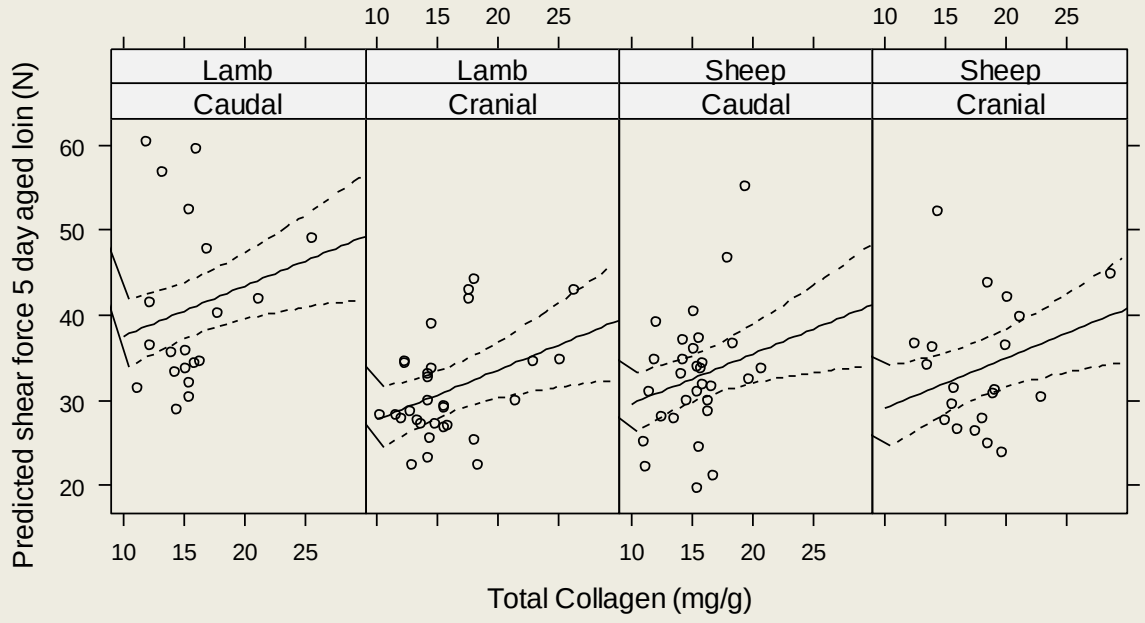

Fig. 28. Relationship between predicted shear force (SF5) and collagen concentrations influenced by the age of the animal (lamb vs sheep) and the position within the LL from which the sample was taken.

When SAXS measures were included in the models there was a positive significant relationship between both SAXS1 (a measure of the 1st lateral spacing between nearest myosin proteins) and SAXS2 (lateral spacing between nearest myosin and actin proteins) and shear force measured at 1 day ($P < 0.01$ and $P < 0.04$, respectively). There was also a significant ($P < 0.01$) interaction between position (cranial or caudal) of the sample in the LL and cell area (which is the hexagonal area between actin and myosin proteins) for SF1 and SF1 had a negative relationship with cell area (Fig. 29).

Fig. 29. Added variable plots for predicted shear force (SF1) against SAXS1, SAXS2 and cell area.

For shear force SF5 there was a significant positive relationship (Fig. 30) with the collagen concentration ($P < 0.005$) and the SAXS1 measure ($P < 0.001$), and the season the animals were killed also impacted on SF5 ($P < 0.04$). Animals killed in Spring were 4.2% tougher than those killed in Autumn. There was also a negative relationship between SF5 and cell area ($P < 0.001$; Fig. 30) and the age of the animal (sheep or lamb) and position (cranial or caudal) of the sample in the LL both impacted on SF5 ($P < 0.02$ and $P < 0.003$, respectively).

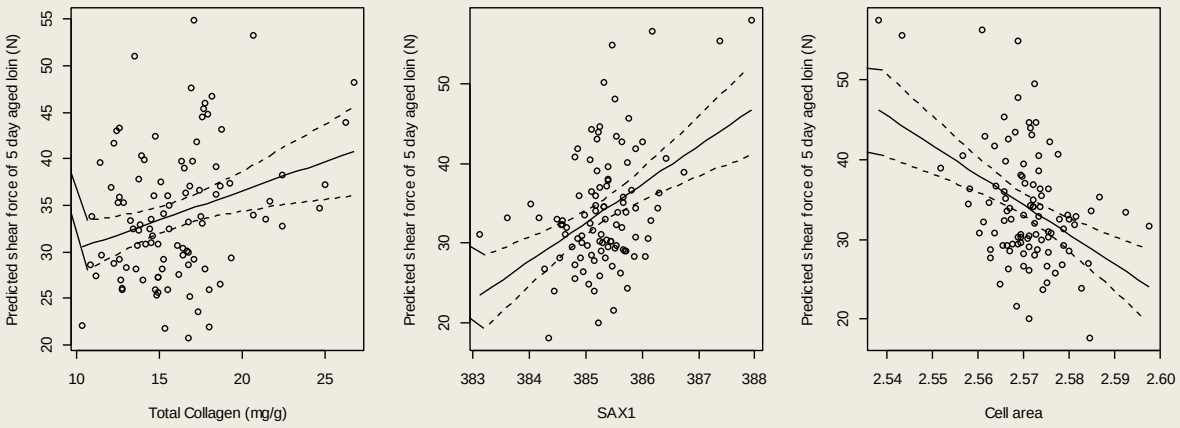

Fig. 30. Added variable plots for predicted shear force (SF5) against collagen concentration, SAXS1 and cell area.

There was a positive relationship ($P < 0.01$) of IMF with GR, and a negative relationship ($P < 0.01$) of IMF with the concentration of soluble collagen. Animal type was also significant with sheep having higher IMF levels than lamb (P= 0.03) (Fig. 31).

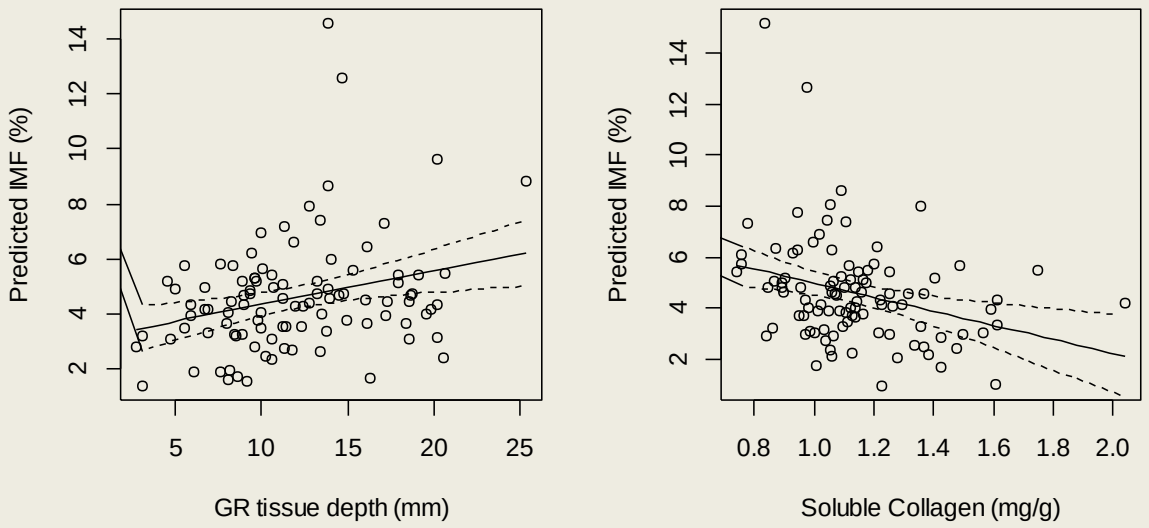

Fig. 31. Added variable plots for the relationship between IMF and GR tissue depth and IMF and the concentration of soluble collagen.

When SAXS measures were included in models to predict IMF, there was a positive interaction ($P < 0.01$) between animal age (sheep or lamb) and GR, and a positive relationship of fat area measured by SAXS (relative measure of fat content; See Fig. 32). There was also a relationship with kill season and IMF percentage with animals killed in Spring having lower IMF% compared to those killed in Autumn.

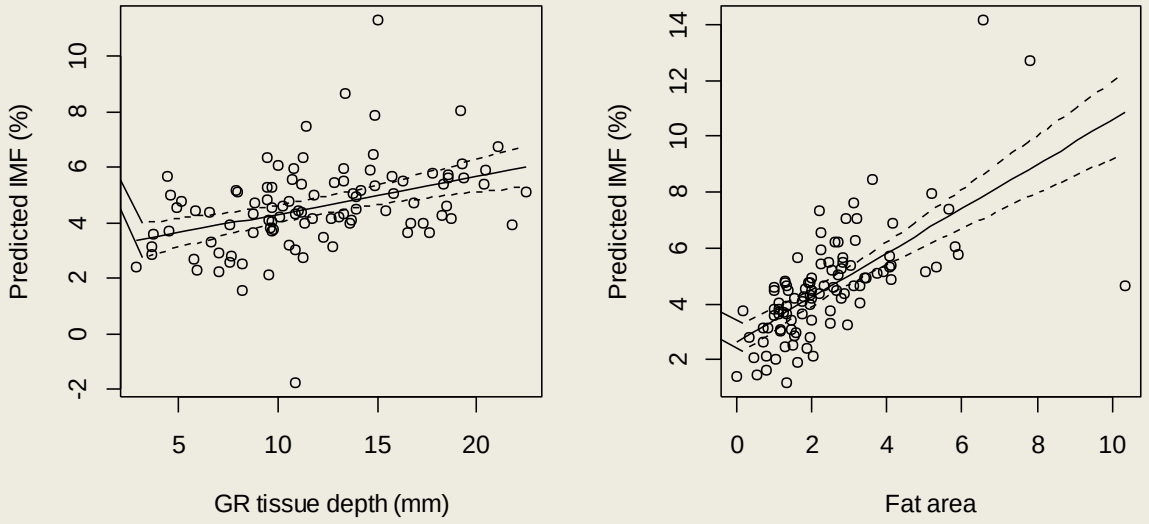

Fig. 32. Added variable plots for the relationship between IMF and GR tissue depth and SAXS fat area.

Finally, there was an effect of position of muscle samples collected (slice A-D) on some of the values measured by the SAXS, with the most cranial slice (Slice A) being consistently different ($P < 0.01$) to the other three slices (B, C, D) for Cell area, SAXS1 and SAXS2.

5 Discussion

5.1 Bioimpedance spectroscopy

Bioelectrical impedance analysis is a technique in which electrical properties of biological tissue is modelled to provide values for parameters that can be interpreted as characterising the anatomical and physiological properties of the tissue. The most widely accepted model describing the impedance of biological tissue is the Cole model (Figs. 6 and 7). This model can provide measures of intra- and extra-cellular water and from these predictions of fat-free mass may be made. The present study demonstrated strong relationships between measured impedance and FFM estimated from dry matter and fat content measurements. FFM in muscle samples could be predicted with similar levels of accuracy and precision to prediction of whole body FFM in live animals and humans (Kyle et al., 2004). Of particular note is that correlations found in the present study are markedly better than those found in previous studies in whole carcasses, for example r = 0.5 to 0.8 (Altmann et al, 2004) compared to 0.94 found here. Furthermore, these results were obtained when prediction of FFM was based on the fundamental model of Hanai (Matthie, 2005) unlike previous studies in which empirically-derived multiple regression equations have been used (e.g. Slanger et al., 1994; Hegarty et al., 1998; Altmann et al., 2004).

Correlations were a little poorer for topside muscle samples than for loin samples. This is due to two main factors. Firstly, meat cut sizes were smaller for topside than for loin samples providing shorter inter-electrode distance. Strength of correlations was found to vary with inter-electrode distance. A number of factors contribute to this lower impedance values decreasing the signal to noise ratio, inhomogeneity of smaller measured volumes and a more rectangular than oblong or cylindrical geometry. Secondly, muscle tissue is anisotropic due to fibre orientation. Ideally, impedance measurements for compositional analysis should be performed along and parallel to the fibre axis. This is difficult to achieve in small rectangular topside cuts. Consequently, impedance measurements are a composite of parallel and perpendicular measurements dependent upon the overall fibre orientation between the sense electrodes. This will vary between samples and contribute to the variation observed in prediction of FFM.

Two approaches to improve measurement precision may be envisaged. One, to decrease electrode spacing such that the measured volume is small, <10 ml. This “caged electrode” approach could provide a single probe that could be readily orientated with respect to fibre direction. The alternative, is exactly the opposite, to lengthen the inter-electrode distance such that the effects of localised variation in fibre orientation are minimised. This is the approach used when impedance measurements have been performed on whole carcasses (e.g. Hegarty et al., 1998). Further work, is required to determine which of these approaches is likely to be most successful.

While FFM could be well predicted, fat mass was poorly predicted. This is due to fat mass % being very low in these meat cut samples. Despite the claims of some impedance device manufacturers, impedance systems do not measure fat. Fat mass is determined indirectly as the difference between weight and FFM. Consequently, relatively small and acceptable errors in prediction of FFM propagate to be unacceptably large proportional errors when calculating fat mass. However, the fat mass of whole or half carcasses or large meat cuts where overall fat mass percentage is higher may be able to be predicted with greater accuracy. Further work is required.

Meat quality encompasses more than simply the relative amounts of fat and lean. The use of impedance to characterise meat quality is underexplored. Electrical techniques have been used to a limited extent to monitor water-holding capacity in pork but have been largely

limited to simple conductivity measurements (e.g. Lee et al., 2000). The present study used state of the art bioimpedance spectroscopy which provides data that better characterises the tissue (see Fig. 7). However, to date application of this has been limited to differentiating tumorous tissue from normal (Laufer et al., 2012) or to monitoring tissue ischemia (Casa et al. 1999). The present study has demonstrated the feasibility of using BIS for tissue characterisation. The challenge is to define which impedance parameters provide information relevant to meat quality. While changes in membrane capacitance may be of value, unfortunately, the meat samples analysed in this study were of similar quality and do not provide sufficient differentiation to allow clear delineation of impedance parameters. Again further work is required and should include a wider range of meat qualities.

5.2 Synchrotron SAXS technology

5.2.1 Relationship between predictors and shear force

Small-angle X-ray scattering is an X-ray diffraction-based technique where a narrow collimated beam of X-rays is focused onto a sample and the scattered X-rays are recorded by a detector (Changizi et al., 2008). This pattern of scattered X-rays carries information on the molecular structure of the material, where myofibril within a muscle fiber has been the focus of this study. Details on the fibril structure of muscle fibres such as myosin and actin, along with a scattering pattern of relative fat content have both been acquired from the SAXS images.

Shear force measured at 1 day was found to be impacted by both SAXS1 and SAXS2 measures which were a measure of the 1st lateral spacing between nearest myosin proteins and the lateral spacing between myosin and actin proteins respectively. As the spaces between the fibres became larger, the shear force results showed the samples to be more tender. It has been previously found that shear force is more closely related to the myofibrillar structure of muscle tissue than to the connective tissue components of meat (Bouton et al., 1975; Bouton & Harris, 1972). Previous work using the SAXS has indicated that the distance of myofibril spacing was linked to drip loss in pork (Diesbourg et al., 1988), which is supported by the fact that around 80% of the water held within living muscle is found in the myofibrils, between the thick and thin filaments (Offer et al., 1989). The result in the current study indicates that there may also be tenderness effects caused by the differences in the myofilament spacing, due to larger spacing resulting in less fibrils in a section than one with smaller spacing.

It is noteworthy that muscle from the sheep had smaller mean equatorial distances than the lamb for both SAXS1 and SAXS2. An explanation for this difference in spacing is that as an animal matures to slaughter weight, the muscle fibres undergo hypertrophy reducing the distance between fibres. For shear force measured at 5 day, only SAXS 1 was significant, indicating a possible effect of ageing on myofibrils and thus the lateral spacing between myosin and actin proteins (SAXS2). The cell area, which is the hexagonal area between actin and myosin proteins, also had a negative impact on shear force measured at 1 day and 5 day and this reflects the fact that SAXS1 is used in the calculation of cell area. While pH was found to have an effect on shear force, the other ‘traditional’ indicators of meat quality such as PS and sarcomere length were found to have no effect on shear force values for LL aged for one day. This conflicts with a number of studies, which have found links between these factors at this time post slaughter (Purchas, 1990; Devine et al., 1993; Chrystall and Daly, 1996).

There were a number of samples that couldn't be measured for sarcomere length and this may be why there was no significant relationship found between shear force and sarcomere length. However, often sarcomere length only explains a small amount of the variation in shear force as illustrated recently (Starkey et al., 2015). PS is a measure of the muscle myofibrillar protein degradation and previous studies have recorded a positive correlation of this with shear force (Karumendu et al., 2009; Starkey et al., 2015). Despite this, there was no relationship between PS measures and shear force in the current study, but it must be noted that the predictors (terms in the model) which are used will impact on which are significant and it appears other predictors were more important in the current study (eg. pH).

Both shear force and the SAXS values indicated that there are structural and chemical differences in meat quality along the length of the longissimus lumborum muscle. There was a difference in shear force values based on whether the sample had been taken from the caudal or cranial end of the muscle. The cranial samples were significantly more tender than the caudal samples, indicating differences in the structure and composition of the muscle along its length. Hopkins and Thompson (2001) looked at the relationship between tenderness, proteolysis, muscle contraction and the dissociation of actomyosin and found a significant difference within muscles depending on the area sampled. They concluded that the portion effects may in part be explained by the ease with which actomyosin could be dissociated, although this effect was not large enough to impact on shear force over and above the effects of proteolysis and fibre density (Hopkins and Thompson, 2001)

For the SAXS traits, one of the sample sites (Slice A) was found to be consistently different for the traits examined (cell area and fat area) further indicating that there are structural and chemical changes across the LL muscle. This finding impacts on the future use of the SAXS beamline, as the position that a sample is taken could cause variation within an experimental design. This differing sample was the most cranial slice taken from the LL muscle and this is something that would have a bearing on the use of synchrotron data.

5.2.2 Relationship between predictors and intramuscular fat (IMF) content

The interaction between GR and animal age for IMF indicated that the older animals were generally leaner than the lamb, but had the highest IMF levels. When compared to other studies it was found that IMF was higher in older animals also, but was early maturing relative to total carcase fat (Pethick et al., 2005; McPhee et al., 2008). A study by Johnson et al. (1972) also suggested that IMF was relatively early maturing when it came to the deposition of fat within a carcase, which was supported by the results of Ponnampalam et al. (2007), but the levels are usually higher in older animals (e.g. Pethick et al., 2005). Animals slaughtered in autumn had higher IMF levels than those slaughtered in spring, reflecting the feed availability of the seasons. During late summer and autumn seasons animals are supplemented with grains and feedlot high in energy due to scarcity in green pasture that are generally low in energy. The high energy diets offered to animals in autumn might have attributed to greater levels of IMF compared with spring animals in the current study. Previous studies have also shown effects of season on IMF content, although this seasonal effect is due to the changing diets of the animals, with concentrates being fed out when there is little feed available (Zikhona et al., 2014).
There was a strong relationship between IMF and the fat area measured by the synchrotron, indicating it is a good measure of IMF content. The previous study by Kosanetzky et al. (1987) indicated that the SAXS could produce a diffraction pattern for different tissue types such as muscle and fat, which was clearly seen in the SAXS results in the current study. The

clear fat peak produced by samples could be measured and this gave a relative measure of IMF content between carcases, which has been shown to be directly related to the IMF and fat content of carcases.

6 Conclusions/Recommendations

6.1 Bioimpedance

- The study has demonstrated the feasibility of impedance spectroscopy measurements. The technique is rapid; a measurement takes less than 1 second and results can be immediately available.
- The procedure is applicable across the range from whole carcasses to individual primal cuts.
- It is non-destructive procedure that could be automated, eg. robotic positioning of electrodes. It is a technique amenable to use on hanging carcasses, i.e. “on the chain” in an abattoir.
- At present, its use would be justified for fat-free mass (lean content) determinations. In order to use impedance to characterise meat quality requires further research.
- Furthermore, the current work indicates that bioimpedance technology could be applicable online to predict fat-free mass or lean content of primal cuts or entire carcasses. Further research is needed to test the practicality of bioimpedance technology to predict composition of primal cuts or entire carcass online at processing sector either as fat-free mass or muscle (lean) content in lamb and beef.

6.2 Synchrotron

- There was a strong relationship found between the myofibrillar (myosin and actin) characteristics detected by synchrotron SAXS technology and a traditional measure of meat tenderness.
- The fat distribution in muscles determined by SAXS technology was positively and strongly related to traditional measure of intramuscular fat content of meat.
- The variation in meat tenderness between cranial and caudal sample positions predicted by traditional measure of WB shear force was also predicted by synchrotron technology such as SAXS1, SAXS2 and cell area for position of sample collected in the muscle and seasons.
- The position effect would need to be considered in any further work, but would be easily managed by an appropriate sampling routine. The findings of this study have shown that the SAXS beamline presents a promising opportunity to determine carcase toughness or tenderness and relative fat content and could be a useful experimental tool, overcoming the need for destructive sampling required for traits like shear force and IMF.
- The current study predicted the differences in actin/myosin structures and fat distribution between positions within a muscle and between seasons. Future study is needed to evaluate the use of synchrotron technology in predicting components such as collagen, fat distribution or myosin/actin levels influencing eating quality traits (eg. tenderness, juiciness etc.) between muscles within an animal and between muscles from different stage of animal growth, which will provide valuable insights to enhance eating quality aspects of lamb and beef.

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