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The effect of muscling EBVs on sheep meat eating quality

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Contents

Executive Summary

- There was a concern that the increased lean associated with using sires with high YEMD or low YFAT Australian Sheep Breeding Values (ASBVs) may result in carcasses with reduced eating quality. The concern arose from evidence that the increased lean in progeny from high YEMD and low YFAT sires was achieved in the live animal by decreased rates of protein degradation which may impact negatively on post-mortem muscle proteolysis in the carcass, resulting in tougher meat. It was important to quantify the impact of variation in ASBVs on eating quality. If their use was associated with reduced eating quality then it was important to investigate mechanisms by which post-mortem proteolysis was accelerated and hence ameliorate the negative effects on eating quality. Tenderstretch and electrical stimulation are both known to accelerate proteolysis and were overlaid at treatments in 2 separate experiments.
- The 109 yearling ewes and wethers from the Sheep CRC/UNE resource flock were used in 2 experiments to investigate the effect of ASBVs on meat quality, in particular the rate of post-mortem proteolysis.
- In experiment 1, a total of 79 Poll Dorset X Merino and Merino progeny which had been grown out to yearlings were slaughtered at a commercial abattoir. Within the Poll Dorset sired progeny there were 11 that were heterozygote for the Carwell gene. All carcasses were stimulated and half tenderstretched with the remainder being normally hung. The *m. longissimus dorsi* (LL) was collected at boning and used to prepare samples for sensory and shear force at 2 and 5 days post-mortem. Samples were also measured for intramuscular fat %, content

sarcomere length, myofibrillar fragmentation index (MFI), degradation of desmin and accumulation of troponin-T products (> 32 kDd).

- In Experiment 2, a total 30 Poll Dorset X Merino and Merino progeny which had been grown out to yearlings were slaughtered at a pet food factory. Within the Poll Dorset sired progeny there were 8 that were heterozygote for the Carwell gene. Carcasses were halved and 1 side stimulated and the other was not stimulated. The *mm. longissimus dorsi* (LL), semimembranosus (SM) were collected at boning and used to prepare samples for shear force at 2 and 5 days post-mortem. The *m. semitendinosus* (ST) was also collected for objective measurements which included sarcomere length, myofibrillar fragmentation index (MFI), degradation of desmin and accumulation of troponin-T products (> 32 kDd).
- Tenderness as assessed by both shear force and sensory showed that progeny from low YFAT sires had tougher meat. MFI values suggested that these progeny also exhibited less post-mortem proteolysis. Similarly sensory and MFI results also revealed that progeny from high YEMD sires had lower sensory scores and less proteolysis during post-mortem ageing. However this was not supported by measures of post-mortem degradation of muscle proteins desmin and troponin-T. Also whilst tenderstretch accelerated the rate of proteolysis early post-mortem it did not interact with ASBVs values. This suggested that either the ASBVs had a minimal effect on the rate of post-mortem proteolysis, or perhaps the methodologies to measure post-mortem proteolysis were not sensitive enough to identify these effects.

- Results from experiment 1 demonstrated a decrease in IMF% with low YFAT and increased YEMD suggesting that it was a potential contributor to the ASBV effects for tenderness. This suggested that the decrease in meat quality through the use of low YFAT or high YEMD sires was to a large degree being driven by changes in IMF%.
- Experiment 2 showed that whilst shear force of the SM increased with increased YEMD, there was no similar trend was evident in the LL muscle. Similarly shear force of the SM muscle increased as YFAT decreased, but again this was not evident in the LL muscle. It is unlikely that the lack of an effect in the LL was simply due to the lower number of animals in experiment 2, as the regression coefficients for the LL were ca. 25% of the magnitude of those reported for experiment 1. In this experiment there were few, or inconsistent, effects of ASBVs on indicators of proteolysis. Electrical stimulation improved the tenderness of both the LL and SM muscles, although there were no interactions between ASBVs and ageing rate response identified for shear force, or for measures of post-mortem proteolysis.
- In both experiments 1 and 2 there was no effect of the Carwell heterozygote on meat quality or measures of proteolysis.

Introduction

The Australian lamb and sheep meat industry has a high priority on delivering carcasses that meet the need for increased weight, less fat and more muscle, whilst maintaining eating quality standards (Pethick, et al 2005). Within the sheep meat industry, producers are rapidly adopting genetic selection tools such as the Australian Sheep Breeding Values (ASBVS), to increase productivity and lift carcass yield through the selection of sires that will produce progeny which have faster growing, leaner, more heavily muscled carcasses.

The net amount of muscle deposited in the body is a function of protein synthesis and degradation. As discussed by Koohmaraie et al. (2002) protein synthesis has no impact on post-mortem meat quality, rather it is the rate of protein degradation that is important. An increased rate of degradation in the live animal will impact favourably on post-mortem meat quality, whilst a decreased rate of degradation will impact negatively on post-mortem meat quality. Previous work has indicated that selection for increased muscling (post-weaning EMD) may be associated with a shift in protein flux towards increased synthesis and reduced degradation, in the lysosomal and proteosomal pathways (McDonough et al., 2006). Increased translation capacity (Greenwood et al., 2006) and muscle cell hypertrophy (McDonough et al., 2006) has also been implicated to increase muscling potential. Results from these studies suggest that continued selection for increased muscling and decreased fatness could lead to reduced eating quality, from both reduced intramuscular fat percentage (IMF%, Hopkins et al,. 2005; Hegarty et al., 2006), and slower protein degradation. Given these scenarios where

production gains could be eroded by the loss in meat quality it is important that the implications of the use of ASBVs on meat quality are clearly understood.

Tenderstretch is known to accelerate post-mortem proteolysis early in the ageing period (O'Halloran et al.; 1998). Therefore, if variation in either yearling growth, fat or muscling breeding values affects the rate of post-mortem proteolysis, the magnitude of the tenderstretch by ageing rate response may be affected. In addition, tenderstretch has the potential to ameliorate any detrimental effects of selection for increased growth, muscling, or decreased fatness, via acceleration of post-mortem proteolysis.

Electrical stimulation technologies have also been shown to produce more tender meat (Hwang et al. 2003, Devine et al. 2006). Whilst the main role of electrical stimulation is the prevention of exposure to cold shortening conditions (Tornberg, 1996, Polidori, et al. 1999, Hwang et al. 2003) in some studies it has also been identified to accelerated the rate of tenderization during ageing (Hopkins and Thompson, 2001; Geesink et al. 1994; Lee et al. 2000, Devine et al. 2006). Given an increased post-mortem proteolysis with electrical stimulation it may provide another tool to ameliorate any detrimental effect on the rate of proteolysis caused by selection for increased growth and muscling, or decreased fatness.

This study investigated the impact of variation growth, muscling and fat ASBV's on meat quality attributes. In particular these experiments provided an opportunity to examine possible interactions between growth, muscling and fatness ASBVs with tenderstretch and

electrical stimulation. Both of these treatments are known to have their effect on meat quality via increased rate of proteolysis.

Materials and Methods

CRC Resource flock

The Sheep CRC resource flock was generated by mating Merino or Poll Dorset rams via artificial insemination to a flock of medium framed merino ewes of similar genetic backgrounds. A total of 17 sires (comprising 8 Merino and 9 Poll Dorset sires) were used. Individual muscling (YEMD), weight (YWT) and fat (YFAT) ASBV's for the 17 sires are shown in Table 1. Some of the Poll Dorset sires used were suspected to be carriers of the Carwell gene, a gene known to cause hypertrophy of eye muscle area. AS discussed below these were genotyped for the presence of the Carwell gene in the heterozygote form.

Experimental design and slaughter, sampling protocol

Animals from the Sheep CRC resource flock were slaughtered in 2 groups. The total flock comprised a total of 109 hoggets (17 months of age) where Merino and Poll Dorset selected to cover a range a ASBVs for muscling (YEMD), fatness (YFAT) and growth (YWT) were crossed with Merino ewes.

The resultant progeny were used in several experiments to investigate the impact of selection for muscling on glycogen metabolism. Experiment 1 used 79 animals which had been used in a glycogen depletion/recovery experiment. All animals sired by Poll Dorset sires were genotyped for the Carwell gene and 11 found to be heterozygote. Experiment 2 used 20 animals (10 merino and 10 Poll Dorset-Merino cross) which had been used in both the same glycogen depletion/recovery experiment and also in an investigation of the effect of selection for

increased muscling on stress sensitivity (catecholamine studies). The other 10 sheep in Experiment 2 were the progeny of sires suspected to be carriers of the Carwell gene. After genotyping, 8 were identified as being heterozygote for the Carwell gene.

Experiment 1: Seventy three hoggets were slaughtered at a commercial abattoir where all carcasses were electrically stimulated and either hung via the conventional hang method (Achilles tendon, AT) or tenderstretched (TS) prior to chilling. All of the Poll Dorset sired animals were genotyped for Carwell; with 11 animals identified as heterozygous for the Carwell gene. Immediately post-mortem (ca. 15 minutes) a section of muscle (ca. 10g) was removed from the causal end of the left m. longissimus thoracis et. lumborum (LL). Upon entry to the chillers all carcasses were stimulated (500 milliamps, for 40 seconds, 68 pulse/second and a pulse width of 1 millisecond) using a CTMS electrical stimulator (Applied Sorting Technology, Australia).

Muscle temperature and pH measurements also commenced upon entry into the chillers. Temperature and pH measurements were taken from the caudal end of the left LL using a MP125 pH meter (Mettler-Toledo, USA). A minimum of 6 readings were made until the carcasses were judged to have reached a pH < 6.0.

In experiment 1 the LL from both carcass sides were removed. Approximately 50 grams of the right loin was kept for the determination of IMF%. Loins were then randomly allocated to ageing treatments at day 2 and 5 post-mortem. The anterior portion of the loins, (ca. 240 mm), was allocated for sensory testing. The remaining loin sample was kept for objective laboratory assessment. Sample blocks were then vacuum

packed and transported packed in ice to either the sensory testing, or objective assessment laboratories. Upon arrival at the respective laboratories the samples underwent further processing. Samples for sensory assessment were prepared at days 2 and 5 post slaughter. At day 2, two 10 gram slices of muscle running parallel to fibre direction were removed from the objective blocks, and wrapped in aluminum foil and frozen (-20ºC) for the determination of myofibrillar fragmentation index (MFI) and sarcomere length. MFI samples were also collected at day 5 post-mortem. In addition, shear force cook blocks (ca. 65 grams) and samples for gel electrophoresis (2-5 grams) were collected at both days 2 and 5. Shear force blocks were frozen at -20ºC until testing whereas gel electrophoresis samples were frozen in liquid nitrogen before being stored at -80ºC. At the conclusion of ageing, at either day 2 or 5 post-mortem, all samples including both sensory and objective samples were frozen at 1500 hours. This was completed to ensure that all samples were aged for the same length of time.

Experiment 2: 30 sheep from the same flock, which were slaughtered and mid-voltage electrical stimulation 500 milliamps, 68 pulse/second and a pulse width of 1 millisecond, CTMS electrical stimulator, Applied Sorting Technology, Australia) applied to the right carcass side. These animals were slaughtered at a local pet food abattoir to allow easy access to carcasses during the chilling period. The m. longissimus thoracis et. lumborum (LL), semimembranosus (SM) and semitendinosus (ST) were removed from both sides and post-mortem proteolysis and meat quality measurements made at 2 and 5 days post-mortem.

In the LL, temperature was measured in the caudal end of the muscle, below the lumbar sacral joint (Temperature probe, MP125 pH meter, Mettler-Toledo, USA). Temperature was also measured in the groove between the SM and ST muscles. In both stimulated and non-stimulated sides approximately 200mg of tissue was excised hourly from the caudal end of the LL and from the SM and ST muscles for pH determined using iodoacetate. In addition, GR depth (total tissue depth over the 12th rib, 110 mm from the midline) was measured.

The day following slaughter, the LLs were dissected and the hind limbs removed from both sides and refrigerated samples transported to the Meat laboratory at the University of New England, Armidale for further processing. The LL muscle was divided into 3 portions (cranial, middle and caudal), whereas the SM and ST muscles were divided in 2 portions (proximal and distal). Within each muscle, sample blocks were randomly applied to ageing treatments (Day 2 and Day 5 post-mortem), with the same sample block/ageing treatment applied to both sides.

A 1cm slice of LL, SM and ST muscle was vacuum packed and stored at 1ºC during for ageing for the collection of electrophoresis samples at day 2 and 5 post-mortem. The slice was taken from the caudal end of the LL and from the middle (proximal – distal axis) of the SM and ST muscles. At day 2 post-mortem muscle samples weighing approximately 2-5 grams were taken from the muscle slice for gel electrophoresis. Electrophoresis samples were frozen in liquid nitrogen, and then stored at (-80ºC) until testing. The remaining muscle slice was re-vacuum packed and stored at 1ºC until the Day 5 sample was collected.

Samples of the LL, SM and ST were also taken for the determination of muscle fibre type and ultimate pH. On day 2 post-mortem, two 5 gram muscle slices running parallel to fibre direction were removed from the LL, SM and ST samples, wrapped in aluminum foil and frozen (-20ºC) for the determination of MFI and sarcomere length. MFI samples were also collected from all muscles at day 5 post-mortem.

Shear force blocks (ca. 65 grams) were collected from the LL and SM muscles at both day 2 and 5 post-mortem (the ST muscle was too small to measure shear force). At the conclusion of ageing shear force blocks were frozen at –20ºC until testing.

Objective Quality Measurements

Shear Force: In both experiments shear force was measured using a Lloyd texture tester (Model LRX, Lloyd Instruments, Hampshire, UL) fitted with a Warner-Bratzler shear blade. The protocol has been outlined by Hopkins and Thompson (2001) and briefly entailed cooking frozen samples for 35 minutes at 70ºC prior to being placed in cold running water for 30 minutes and storing overnight at 4°C. From each cooked sample, 5–6 samples with a cross sectional area of 1cm^2 were prepared for shearing perpendicular to the myofibril direction.

Myofibril Fragmentation Index (MFI); Muscle samples (10g) taken from day 2 and day 5 aged LL (n = 144) held at –20ºC were used for the determination of the MFI based on the methods of Hopkins et al. (2004), however some minor modifications were made. In the present experiment after the third wash samples were finally suspended in 40 ml of cold buffer. Triplicate samples were prepared to a final protein concentration of 0.5 mg/ml. The absorbance of vortexed samples was immediately read at

540 nm. The mean of the triplicate absorbances was determined and multiplied by 150 to give a MFI value.

Sarcomere Length: Sarcomere length was determined using the Helium-Neon light diffraction technique outlined by Perry et al. (2001). A minimum of 4 readings were taken per sample. The mean of individual readings was reported as the average sarcomere length.

Intramuscular fat percentage (IMF%): Percentages of intra-muscular fat were determined using the near infrared (NIR) procedure outlined by Perry et al. (2001).

SDS PAGE and Western Blots: The degradation patterns of muscle proteins desmin and troponin-T were determined based on the methodology described by Thomson et al. (2007), however some minor modifications were made. In the present experiment the protein concentration of samples was determined in triplicate and an internal reference sample was included as a standard on each gel to enable correction for gel processing effects. The degradation bands investigated were at approximately 48kDa for desmin and <31kDa for troponin-T degradation products (Figure 1).

Sensory Taste Panel Assessment

In experiment 1 sensory assessment was determined using the sensory protocol described by Thompson et al. (2005). Briefly, loin steaks were prepared, grilled and presented to untrained consumers who assessed samples for tenderness, juiciness, flavour and overall liking using a 100 point continuous scale from 0 (lowest) – 100 (highest). 10 panelists assessed each sample and scores were averaged to provide final sensory assessment score.

Histology

A 24 h post mortem sample of tissue was removed from the LL immediately fixed in 10% formaldehyde (stock 39% w/v, Sigma, Melbourne, Australia) in 0.01 M phosphate buffered saline, pH 7.3 for 24 h at room temperature. Tissue was embedded in paraffin after dehydration through a graded series of alcohol (70, 80, 90 and 100% reagent grade absolute ethanol, respectively) followed by 2 changes in xylene. Sections, 4.5-6 µm in thickness, were obtained using a microtome, de-paraffinised in xylene and re-hydrated in graded ethanol to water. Sections were placed in Wiegert's iron haematoxylin for 10 min, rinsed in water, and then placed in Van Geison's stain (1% acid fuchsin in saturated picric acid; Sigma-Aldrich, Sydney, Australia) for 5 min. This procedure differentially stains connective tissue seams (red-purple colour) and muscle fibres (yellowish- brown colour). Sections were rinsed rapidly in 2 changes in 100% ethanol, cleared in xylene and then mounted permanently on a slide with a histological mounting medium (Permount, Fisher Scientific, Pittsburgh, PA). The sections were photographed using a Zeiss Axiocam attached to a Zeiss Axiophot microscope and images were stored in jpeg format.

Morphometric analysis

The distribution and thickness of the connective tissue seams were analysed using a custom-written software application in Perl (written by WB). The application makes use of the ImageMagick Perl module. Each image is first reduced to four preselected colours representing muscle, connective tissue, tears and background. Once each image has been subdivided into these representative colours, the analysis is guided by

imaginary concentric circles superimposed on each image. As each circle is traversed, angular coordinates are recorded at any colour change. The circumference of each circle is split into total lengths for each colour. Integrating across all circles provides an estimate of the connective tissue versus all tissue. Area of connective tissue (red) and skeletal muscle (orange) were then assessed at the locations specified by the perimeters of the circles. The area proportion of total muscle that was connective tissue was expressed as the percent connective tissue in each image as determined from the pixel density for each component; muscle and connective tissue. Also from this systematically sampled data the average distance traversed across consecutive connective tissue seams (seam thickness; ST) and the distance between consecutive seams (fascicular width; FW) was calculated. Other variables were computed describing the sum of all connective tissue seam measurements as total seam thickness (TST) and the sum of all fascicular widths as total fascicular width (TFW). To remove any potential bias due to area of tissue section captured the area of each image was also calculated and used as a covariate in analyses. All linear measures in *pixels* were converted to *µm* by a factor of 1.18.

Calculation of temperature at pH 6.0

Exponential rates of decline for pH and temperature were calculated using the PROC NLIN (SAS 1999) where the following function was fitted to the $pH_{t,\ and}$ time data,

$pH_t = pH\mu + (pH_i - pH\mu)e^{-kt}$ with parameters $pH\mu$, pH_i and k estimated.

The above equation was also used to fit the temperature decline data, where tempμ, $temp_i$ and k were estimated. Time at pH 6.0 was calculated and then used to determine Temperature at pH 6.0 (temp@pH6).

Statistical analyses

Measures in both experiment 1 and 2 were analysed using a MIXED model which included terms for fixed effects (animal sex, hang method, ageing period, carcass side, sire breed, and Carwell genotype(sire breed) and co-variates (YEMD, YWT and YFAT, GR, HSCW, and linear and curvilinear terms for temp@pH6.0). Appropriate 1^{st} and 2^{nd} order interactions were tested and non-significant interactions ($P>0.05$) excluded from the analysis. A random term animal(sire*sirebreed*sex*hang method) was included. For the analysis of shear force, sample pre-cook weight was included as a co-variate, to account for differences in sample weights. For the analysis of muscle proteins desmin and troponin-t an internal reference samples was included as a covariate to account for differences between gels.

Variables HSCW and GR tissue depth were analysed with fixed effects (sex, sirebreed and carwell genotype) and co-variates (YEMD, YWT and YFAT). The model for GR tissue depth also contained HSCW as a co-variate.

Results

Experiment 1

Carcass Traits

Table 2 shows the means (± s.d.) and ranges for carcass traits, measures of objective tenderness and sensory assessment scores. HSCW was significantly ($P<0.05$) affected by sire breed, sire YWT, sex, and GR tissue depth. The Poll Dorset progeny had a heavier HSCW than the Merino progeny (21.9 ± 0.5 vs 18.1 ± 0.6 kg HSCW). In addition, increased sire YWT resulted in heavier HSCW by 0.14 ± 0.07 kg for each kg increase in sire YWT ($P<0.05$). HSCW was also affected by sex, with females being lighter than males (19.3kg vs. 20.7kg; $P<0.05$). There was a positive effect of GR on HSCW, with HSCW increasing by 0.18 ± 0.03 kg per mm of GR ($P<0.01$). Whilst HSCW was affected by selection for sire YWT no effect of sire YEMD or YFAT was identified (Table 3, $P>0.05$).

As shown in Table 3, GR was significantly affected by HSCW and sex ($P<0.01$). GR increased by 2.3 mm per kg increase in HSCW and females had higher GR levels than males (20.2 ± 1.5 vs 14.6 ± 1.4 mm). GR depth was not affected by the sire ASBVs ($P>0.05$).

Sarcomere length was significantly affected ($P<0.01$) by a carcass side by sire YWT interaction, as well as by hang method and sire breed ($P<0.05$). In the right carcass side sarcomere length declined by –0.011 ± 0.005 µm for each kg increase in sire YWT ($P<0.05$). The sarcomere lengths in the TS carcasses were 0.07µm longer than those from AT hung carcasses (1.82µm, vs. 1.75µm). At the same HSCW, the Poll Dorset progeny had longer sarcomere lengths than the Merinos (1.85µm vs.

1.71µm, P<0.05). Whilst sarcomere length was affected by selection for sire YWT, there was no effect of sire YFAT or YEMD (P>0.05).

For IMF% there was a significant sire breed by sire YFAT interaction (P<0.01) where for Poll Dorset progeny IMF% increased by 0.47% ± 0.10 per mm increase in YFAT. In contrast, in the Merino progeny IMF% declined by -0.62% ± 0.27 per mm increase in YFAT (Table 3, Figure 2; P<0.01). IMF% levels were also affected by sire YEMD (P<0.01). IMF% declined by –0.27 ± 0.07% for each mm increase in YEMD (Table 3). After adjusting for sire ASBVs the Poll Dorset progeny carrying the Carwell gene had lower IMF% than the non-Carwell animals (2.4 ± 0.2% vs 3.1 ± 0.2%, Table 3; P<0.01). In addition, IMF% was also affected by HSCW and animal sex. IMF% increased by 0.11 ± 0.03% with each kg increase in HSCW (P<0.01) and IMF% was higher in the females (3.22% vs. 2.82%, Table 3; P<0.05).

Objective Meat Quality Traits

The mean and range for objective meat quality traits is shown in Table 2. Due to sub-optimal chilling conditions experienced during this experiment it is likely that all carcasses were exposed to cold shortening conditions as the temperature at pH 6.0 below 10ºC (K. Martin, pers. comm.).

Shear Force

Shear force declined with post-mortem ageing from 74 N at day 2 to 57 N at day 5 (P<0.01). The application of the TS treatment reduced shear force values by approximately 25% (P<0.01). Predicted values for the TS and AT treatments were 56 N and 75 N. Shear force was also affected by HSCW, where it declined by –2.65 ± 0.98N for each kg

increase in HSCW (P<0.01, Table 4). In addition, shear force samples taken from the left carcass side were less tender than those from the right carcass side (70 N vs. 60 N; P<0.01). Length of time between death and electrical stimulation affected shear force (P<0.05); with increasing length of time to stimulation increasing shear force.

Shear force was also affected sire YFAT (P<0.05) with a lower YFAT resulting in a higher shear force (7.86 ± 2.65 Newton for each mm decline in YFAT). To test if the YFAT effect was driven by differences in fat levels, IMF% was included in the analysis as a covariate. The inclusion of the IMF% term was significant (P<0.05) and accounted for a similar proportion of the variance as the YFAT term. In the presence of IMF% the YFAT term also failed achieve significance (P>0.05).

Myofibrillar Fragmentation Index (MFI)

MFI increased with post-mortem ageing, although the rate of change was affected by hang method (P<0.05). Early in the post-mortem ageing period, at day 2, the TS samples had higher MFI values at 33.3, compared with 20.6 for the AT hung carcasses (P<0.05; Table 4). At day 5, there was no difference between hang methods in MFI values (mean value of 43 for both the AT and TS sides, P>0.05).

Sire YEMD also affected MFI (P<0.01) but this response varied across carcass sides (Table 4). In the left carcass side, increased sire YEMD caused MFI to decline by –6.54 ± 1.60 units for each mm increase in YEMD (Table 4; P<0.01), whereas, in the right carcass side the size of this response was smaller as MFI declined by –3.41 ± 1.55 units for each mm increase in YEMD (P=0.063). In addition, sire YWT also affected MFI, where it declined by -0.89 ± 0.37 units for each kg increase in sire YWT

(Table 4; P<0.05). Conversely, MFI increased by 5.48 ± 1.94 for each mm increase in sire YFAT (P<0.05).

In TS carcasses MFI increased with HSCW, with a 2.62 ± 0.69 unit increase in MFI / kg HSCW, but this trend was not observed in the AT carcasses (P>0.05). MFI declined as length of time between death and electrical stimulation increased (P<0.05).

Desmin degradation

Levels of native desmin declined with post-mortem ageing although the rate of degradation differed between carcass hang treatments (P<0.05, Table 4). At 2 day post-mortem the TS samples had approximately 32% less native desmin than the Achilles hung samples (P<0.01). At 5 days post-mortem there was no difference between TS and AT hang treatments (P>0.05). In TS carcasses, levels of native desmin declined as HSCW increased (P<0.01), however this effect was not apparent in AT carcasses (P>0.05).

Levels of native desmin increased as the sire YWT ASBV increased (Table 4; P<0.05). Likewise, in the right carcass side levels of native desmin increased as sire YFAT decreased (P<0.05). There was no apparent effect of sire YFAT in the left carcass side (P>0.05, Figure 3). Whilst sire YWT and YFAT were identified to affect the degradation pattern for desmin, no effect of sire YEMD was identified (P>0.05).

Troponin-T degradation products

Levels of troponin-T degradation products (<31kDa) increased during post-mortem storage with predicted volumes doubling between day 2 and day 5 (P<0.01). In TS carcasses, the amount of troponin-t

degradation products was also affected by HSCW (P<0.01); where the volume of degradation products increased as HSCW increased. However, this effect was restricted to the TS carcasses.

Sire YFAT also affected the level of troponin-t degradation products, although this effect was only observed in the right carcass side (P<0.05, Table 4, Figure 4), where the level of degradation products declined as sire YFAT ASBVs declined. Whilst lower sire YFAT reduced the levels of troponin-t degradation products, no effect of sire YEMD or YWT was observed (P<0.05).

Sensory Traits

Sensory scores for all traits (tenderness, juiciness, flavour and overall like) were influenced by a variety of factors. Scores for all traits increased with post-mortem ageing (Table 5 & 6; P<0.01). The application of the TS treatment also improved sensory scores for all traits by between 7 – 10 % (Table 6; P<0.05).

In addition, sensory scores were affected by sire YFAT and YEMD (P<0.05). Lower sire YFAT caused sensory scores to decline for tenderness, juiciness and overall like (P<0.01) and flavour traits (P<0.05). The regression co-efficients for these effects are shown in Table 7. Likewise, increased sire YEMD caused a decline in tenderness, juiciness and overall like (P<0.01) and flavour (P<0.05).

To investigate whether these effects were caused by changes in fat levels, IMF% was included as a covariate, which caused the YFAT term to become non-significant (P>0.05) for both the juiciness and flavour traits and the YEMD term to become non-significant (P>0.05) for all sensory traits except juiciness. Likewise, it reduced the F-ratios for the tenderness

and overall liking traits for YFAT and the F-ratio for the juiciness trait for YEMD, leading to both a reduction in the level of significance and the size of the regression co-efficients (Table 6).

HSCW also affected both the flavour and overall like scores ($P<0.05$). Flavour scores increased by 0.95 ± 0.37 points and overall liking scores increased by 0.92 ± 0.40 points for each kg increase in HSCW ($P<0.05$).

Fascicular structure

There was no significant ($P>0.05$) effect of yearling ASBVs on variation in percent connective tissue. Also variation in total perimysial seam thickness was not associated with tenderstretch treatment, sex or breed. There was a breed effect on average perimysial seam thickness, whereby the Poll Dorset sired animals had significantly ($P < 0.01$) thinner perimysial seams in the LL than Merino sired animals. There was no association between total or average fascicular width and ASBVs, no any effect of breed, sex or tenderstretch treatment on total or average fascicular width.

Experiment 2

Liveweight and Carcass Traits

Table 7 shows raw means (± s.d) and ranges for liveweight, HSCW, and GR for the animals slaughtered in experiment 2. Progeny from Merino sires were lighter (P<0.01) than Poll Dorset sired animals (36.9 ± 1.6 kg vs. 49.2 ± 1.1 kg). For Poll Dorset progeny only, variation in sire YWT affected HSCW, where HSCW increased by 0.45 ± 0.16 kg for each kg increase in sire YWT (P<0.05, Table 8). GR tissue depth was not affected by variation in sire ASBVs or sire breed (P>0.05, Table 8).

Objective meat quality assessments

Sarcomere Length (SL)

Sarcomere length (SL) was affected by variation in sire YEMD, with SL decreasing by –0.03 ± 0.01 µm per mm increase in sire YEMD (P<0.01, Table 10). Females had longer sarcomere lengths than males (2.09 ± 0.02 vs 2.02 ± 0.02, P<0.01). The ST muscle had longer sarcomere length than both the LL and SM muscles (P<0.01). Predicted means for the ST, LL and SM muscles were 2.40, 1.90 and 1.88 (average se ± 0.02µm) respectively. In the LL muscle, longer sarcomere lengths were recorded in the caudal portion (P<0.01). Likewise, in the ST muscle longer sarcomere lengths were recorded in the distal portion (P<0.01).

Shear Force

In the SM muscle shear force increased by 0.46 ± 0.17kg for each unit increase in sire YEMD (P<0.01, Figure 5), although no effect was observed in the LL muscle (P>0.05). Shear force was affected by a sire YFAT by muscle interaction (P<0.01). Whilst there was no effect of

variation in YFAT in the LL muscle (P>0.05), in the SM muscle there was a trend (P=0.07) for shear force to decline as YFAT increased. Electrical stimulation lowered shear force values in both muscles by 0.47 ± 0.15 kg (P<0.01), but did not affect the rate of post-mortem ageing (P>0.05). In both the LL and SM, shear force declined by approximately 1.2 kg between day 2 and day 5 (P<0.01). Shear force declined by –0.22 ± 0.11 kg for each kg increase in HSCW (P<0.05). There was a curvilinear relationship between temp@pH6 and shear force with a minimum shear force in the LL and SM occurring at 17.6°C temp@pH6 (Table 10, Figure 6).

Cook Loss

Within the LL muscle, cook loss percentage (CL%) increased from the cranial to caudal positions with predicted means of 18.9, 21.1 and 24.0% (average se ± 0.5%) for the cranial, middle and caudal positions respectively (P<0.01). Similarly in the SM, position effects showed the proximal position having lower cook loss values (19.1 vs 20.1 ± 0.5% for the proximal and distal positions respectively, P<0.01). The LL muscle had higher cook loss percentages than the SM muscle at day 5 post-mortem (P<0.01). In the SM only, CL% decreased by 2.2% between day 2 and 5 post-mortem (P<0.01).

Sample pre-cook weight affected CL%, where CL% declined by –0.24 ± 0.024% per gram increase in pre-cook weight (P<0.01). Temperature at pH 6.0 (curvilinear term) also affected cook loss percentage with a point of inflexion, corresponding to a minimum cook loss percentage being identified at 15.2°C (P<0.05). Variation in sire ASBVs did not affect CL% (Table 10; P>0.05).

Myofibrillar Fragmentation Index (MFI)

In the electrically stimulated sides, MFI declined by -2.90 ± 1.37 units per mm increase in sire YEMD, but this trend was not observed in the non-stimulated sides (P>0.05). MFI was also affected by variation in YFAT, although the size of the effect was moderated by the application of electrical stimulation (P<0.05). In non-stimulated sides MFI declined by –16.49 ± 6.17 units per mm increase in sire YFAT (P<0.01), whereas in stimulated sides MFI declined by –13.49 ± 6.17 (P<0.05).

There was an interaction between sire breed and YWT (Table 10, P<0.05) on MFI. For the Merino sire progeny, MFI increased by 1.23 ± 0.43 units per kg increase in YWT. In contrast, in the Poll Dorset sired animals there was a trend (P=0.06) for MFI to decline by –1.52 ± 0.80 units per kg increase in YWT.

Figure 4 shows that at both days 2 and 5 post-mortem MFI declined as sire YFAT value increased, although the rate of increase was greater at day 5 post-mortem (P<0.05 – Figure 7).

MFI was also affected by a YFAT by sire breed interaction (Table 10; P<0.01). Figure 8 shows that for the Merino sired progeny, MFI declined as sire YFAT increased, although there was a smaller range in MFI. The regression co-efficients were –13.70 ± 3.37 MFI units per mm increase in YFAT for the Merino sired progeny (P<0.01) and +3.98 ± 1.96 MFI units per mm increase in YFAT for the Poll Dorset sired progeny (P<0.05).

Figure 9 shows that in the SM and ST muscles the Poll Dorset sired offspring had lower MFI values (P<0.01). No sire breed effect was identified for the LL muscle (P>0.05). In addition, Figure 9 highlighted a trend for the Merino sired animals to have higher MFIs, although this

trend only achieved significance in the ST muscle (P<0.05). At days 2 and 5 post-mortem the LL muscle had higher MFI values than both the SM and ST muscles (P<0.01, Table 10). In addition, at day 2 and 5 post-mortem the SM muscle had higher MFI values than the ST muscle (P<0.05). In the SM muscle only, the proximal position recorded higher MFI values (40.16 ± 2.26 vs 30.09 ± 2.26, P<0.01). No positional differences were identified in the LL or ST muscles. MFI was also affected by HSCW, where MFI increased by 3.00 ± 0.87 units per kg increase in HSCW (P<0.01).

Desmin

In the Merino sired progeny only, levels of native desmin increased (P<0.01, Table 10) as sire YEMD increased. The level of native desmin was also affected by a sire YWT by electrical stimulation interaction (P<0.05), the regression co-efficients differ in direction for the electrically stimulated and control sides, although neither regression co-efficient was significantly different from zero (P>0.05).

Figure 11 showed that levels of native desmin were affected by muscle by ageing rate interaction (P<0.01). At day 2 post-mortem there was little difference in native desmin levels between all three muscles (P>0.05). However at day 5 post-mortem, the LL muscle had significantly less native desmin than both the SM and ST muscles (P<0.01), indicating a greater rate of protein breakdown during post-mortem storage.

Temperature at pH 6.0 (curvilinear term) affected levels of undegraded desmin with a point of inflexion, corresponding to the lowest levels of desmin was identified at 14.6°C.

Troponin-T

In the Poll Dorset progeny only, the level of troponin-t products declined as sire YWT increased ($P<0.05$), indicating a reduction in the rate of troponin-t degradation. The level of troponin-t degradation products was also affected by a sire YWT by muscle interaction, where in the SM muscle only, the level of troponin-t degradation products increased as sire YWT increased ($P<0.01$).

At day 2 post-mortem levels of troponin-T degradation products (<30kDa) did not differ between the 3 muscles ($P<0.05$). However, at day 5 post-mortem the LL muscle had significantly greater level of troponin-t degradation products than the SM and ST muscles ($P<0.01$). At day 5 post-mortem the SM muscle also had higher levels of troponin-T degradation products than the ST muscle (Table 10; $P<0.01$).

The level of troponin-t degradation products was also affected by a muscle by sire breed interaction ($P<0.05$). In the Poll Dorset sired progeny, the LL had higher levels of troponin-t degradation products than both the SM and ST muscles ($P<0.01$). Troponin-t degradation product levels did not differ between the SM and ST muscles ($P>0.05$). Similarly, in the Merino sired progeny the LL muscle had higher levels of degradation products than the ST muscle ($P<0.01$), but was not different to the SM muscle ($P>0.05$). Likewise, the level of troponin-t degradation products did not differ between the SM and ST muscles in the Merino sired progeny ($P>0.05$). The level of troponin-t degradation products was also affected by HSCW, with levels of degradation products increasing with HSCW ($P<0.05$).

Discussion

Experiment 1

Tenderness as assessed by both shear force and sensory showed that progeny from low YFAT sires had tougher meat. In addition the MFI values suggested that progeny from low YFAT sires exhibited less post-mortem proteolysis. Similarly sensory and MFI results also revealed that progeny from high YEMD sires had lower sensory scores and less proteolysis during post-mortem ageing. These results suggested that use of low YFAT and high YEMD sires, had a detrimental affect on product quality.

Whilst the MFI results suggested that the sire YFAT and YEMD affected the rate of post-mortem proteolysis, subsequent investigation of the degradation of muscle proteins desmin and troponin-t failed to identify similar effects. Both the MFI and desmin data indicated the TS response accelerated the rate of proteolysis early post-mortem which is in accordance with O'Halloran et al. (1998). However if sire YFAT and YEMD were impacting the rate of post-mortem proteolysis it could be expected that they would also interact with the magnitude of the TS response. Contrary to this expectation this experiment did not show an interaction between hang treatment and ASBVs for tenderness measurements. This suggested that either the ASBVs had a minimal effect on the rate of post-mortem proteolysis, or perhaps the methodologies to measure post-mortem proteolysis were not sensitive enough to identify these effects.

Increases in IMF% have been associated with improved tenderness and palatability levels in sheep (Pethick et al 2005) and beef (Thompson 2004). Results from this experiment demonstrated a decrease in IMF%

with low YFAT and increased YEMD suggesting that it was a potential contributor to the ASBV effects for tenderness. Certainly for shear force, adjustment for IMF% removed the effect of YFAT. Similarly for sensory, adjustment for IMF% either removed or decreased the effect of YFAT and YEMD on meat quality. This suggested that the decrease in meat quality through the use of low YFAT or high YEMD sires was to a large degree being driven by changes in IMF%.

Interestingly, the effect of selection for YFAT on IMF% varied between sire breeds, with IMF% decreasing with low YFAT Poll Dorset sires and increasing with low YFAT Merino sires. This potentially indicated a difference in fat partitioning effects between the Poll Dorset and Merino sire breeds. Fat partitioning differences between sheep breeds have previously been reported (Thompson 1990), but as this was not measured in this study and there was no effect of YFAT on GR or HSCW, it remained unclear what caused the effect in the present experiment. IMF% also declined as sire YEMD increased. This is in agreement with the findings of Hopkins et al. (2005), where IMF% declined by –0.11 ± 0.06 % per mm increase in post-weaning EMD, although the size of the regression co-efficient in this experiment was almost double that previously reported.

Unlike the sire YEMD and YFAT terms, no detrimental (or favorable) effect of sire YWT was identified by shear force or sensory assessment for either sire breed. This was consistent with Hopkins and Hegarty (2004) Hopkins et al. 2005) where no effect of increasing growth potential on meat quality was observed. However, both the MFI and desmin degradation results indicated that increased YWT slowed the rate of post-

mortem proteolysis, which potentially would be expected to impact on post-mortem meat quality.

The sensory, shear force, IMF% and the MFI results indicated that increasing sire YEMD and decreasing sire YFAT were both having an effect on eating quality and post-mortem proteolysis. However there was a significant correlation between the YFAT and YEMD values for the sires used in this experiment ($r = 0.58$, $P<0.05$). Importantly, the presence of this correlation may have inflated the magnitude of the YFAT and YEMD effects. This was demonsrated by the regression coefficient between YFAT and IMF% being twice that reported by Hopkins et al. (2005). Therefore, it was imperative that where both terms were significant, the size of the regression co-efficients for YFAT and YEMD need to be treated with caution.

TS improved product quality by restricting the ability of muscles in the loin and hindlimb from contracting during the onset of rigor (Thompson et al. 2005b). Whilst TS improved product quality the magnitude of the response can be quite variable, with larger responses observed under rapid chilling conditions (Thompson et al. 2005b). Results for MFI and desmin degradation indicated that the tenderstretch response initially increased the level of postmortem proteolysis early in the ageing period, which agrees with O'Halloran et al. (1998). Sensory scores of meat in experiment 1 were low and shear force at days 2 and 5 higher than the general threshold of ca. 50 N (5kg) generally accepted to indicate tough meat (Hopkins et al. 1998; Shorthose et al. 1986). The increased toughness in this experiment presumably resulted from a combination of a sub-optimal chilling environment at the processing plant,

(where loin muscles entered rigor mortis at temperatures of less than 6ºC Martin pers. comm.) and the low (1ºC) post mortem ageing temperatures.

Whilst mid-voltage stimulation was applied to all carcasses upon chiller entry, this did not avoid the cold shortening scenario, which was confirmed by a relatively short sarcomeres. Interestingly sarcomere length did not vary between hang treatments possibly because of pre-rigor sampling close to where sarcomere length was measured. Although the carcasses were electrically stimulated, the time from death to stimulation may also have reduced the effectiveness of the stimulation procedure.

Minimal effects of the effect of the Carwell gene were identified in the present experiment, however this experiment only contained animals that were heterozygous for the gene. In addition to the sire ASBV effects, carriers of Carwell gene had heavier carcass weights and lower levels of IMF% than non-carriers. No effect of the Carwell gene was identified by either sensory or objective measures.

Experiment 2

This experiment showed that shear force of the SM increased with increased YEMD, although no similar trend was evident in the LL muscle. Similarly shear force of the SM muscle increased as YFAT decreased, but again this was not evident in the LL muscle. Experiment 1 identified that intramuscular fat % was the main driver of YFAT and YEMD effects on toughness. Unfortunately, this could not be determined in the present experiment as there was not sufficient sample. It is intriguing that the ASBVs effects were not evident for the LL muscle (whereas they were in experiment 1). This could not be explained simply by the lower numbers

in experiment 2, as the regression coefficients for the LL were ca. 25% of the magnitude of those reported for experiment 1.

Similarly there were few, or inconsistent, effects of ASBVs on indicators of proteolysis. There was a YFAT by ageing rate response which indicated that levels of proteolysis increased as sire YFAT decreased. It was somewhat puzzling that the magnitude of this response was greater after the conclusion of post-mortem ageing and no reason can be put forward to explain this. Similarly there was a YFAT by stimulation treatment interaction for MFI, whereby progeny from low YFAT sires had a greater rate of proteolysis in unstimulated compared to stimulated sides, although this effect was not evident in either disappearance or accumulation rates of the indicator proteins.

The effect of YEMD interactions on indicators of proteolysis was just as variable. Stimulated sides still showed a trend for less proteolysis with higher YEMD sires, whereas there was no effect on control sides. As already mentioned the YEMD effect was not evident for shear force, nor for troponin-T. For desmin there was a positive effect of YEMD, which showed higher proteolysis associated with use of high YEMD sires.

The use of electrical stimulation has been associated with the acceleration of tenderization during post-mortem ageing (Hopkins and Thompson, 2001; Devine *et al.,* 2006). In this experiment a safer mid-voltage stimulation treatment (Shaw et al. 2005) was applied to the right carcass side in an attempt to accelerate the rate of post-mortem proteolysis and enable examination of possible interactions with ASBVs. Whilst the electrical stimulation treatment improved the tenderness of both the LL and SM muscles, no interactions between ASBVs and ageing

rate response identified for shear force, or for measures of post-mortem proteolysis.

As with experiment 1 there was a significant correlation between the YFAT and YEMD breeding values of the sires used in this experiment was identified ($r = 0.58$, $P<0.05$). Importantly, the presence of this correlation may have led to an overestimation of both the YFAT and YEMD effects. Therefore, it is imperative that where both terms are significant, the size of the regression co-efficients for YFAT and YEMD need to be treated with caution.

As for experiment 1 the current analysis showed minimal effects of the Carwell gene on meat quality or measures of proteolysis. It needs to be mentioned that the Carwell effect (or lack of effect refers to heterozygote animals rather than homozygotes.

Conclusion

This study showed that ASBVs for YEMD and YFAT were associated with increased sensory toughness and shear force of the *m. longissimus dorsi* in experiment 1, However in experiment 2 where only shear force was measured on 2 different muscles this association was only evident in the *m. semimembranosus*. In experiment 1 this decrease in quality was largely driven by decreased IMFAT%, however it was not possible to test the contribution of IMFAT% as it was not measured in Experiment 2 due to insufficient sample.

There was a trend for ASBVs for YEMD and YFAT to be associated with decreased post-mortem proteolysis but this varied for the different measures of proteolysis. In experiment 1 MFI values supported a decreased proteolysis in progeny from high YEMD sires, although the same trends were not evident for desmin degradation and troponin-T accumulation. This may have reflected the variability in measures of protein degradation. In experiment 2 there were few, or inconsistent, effects of ASBVs on indicators of proteolysis.

Whilst both tenderstretch and electrical stimulation improved the tenderness and appeared to increase proteolysis in both experiments, there were no interactions between ASBVs and ageing rate response.

There was no effect of ASBVs on measures of connective tissue structure. Also in both experiments there was no effect on the Carwell heterozygote on meat quality or proteolysis.

References

Bouton PE, Fisher AL, Harris PV, Baxter RI (1973) A comparison of the effects of some post-slaughter treatment on the tenderness of beef. *Journal of Food Technology*. 8, 39-49.

Devine CE, Lowe TE, Wells RW, Edwards NJ, Hocking Edwards JE, Starbuck TJ, Speck PA (2006) Pre-slaughter stress arising from on-farm handling and its interactions with electrical stimulation on tenderness of lambs. Meat Science, 73. 304-312.

Geesink GH, van Laack RL, Barnier VMH, Smulders FJM (1994). Does electrical stimulation affect the speed of ageing or ageing response. Sciences Des Aliments, 14, 409 – 422.

Greenwood, P.L., Davis, J.J., Gaunt, G.M. and Ferrier, G.R. (2006) Influences on the loin and cellular characteristics of the *m. longissimus lumborum* of Australian Poll Dorset-sired lambs. *Australian Journal of Agricultural Research.* 57, 1– 12.

Hall. D. G., Gilmour, A.R., Fogarty, N.M., and Holst, P.J (2002) Growth and carcass composition of second cross lambs. 2. Relationships between estimated breeding values of sires and their progeny performance under fast and slow growth regimes. *Australian Journal of Agricultural Research.* 53, 1341 – 1348.

Hegarty, R.S., Shands, C., Marchant, R., Hopkins, D.L., Ball, A.J and Harden, S. (2006) Effects of available nutrition and sire breeding value for growth and muscling on the development of crossbred lambs. 1. Growth and carcass characteristics. *Australian Journal of Agricultural Research* 57, 593-603

Hopkins, D.L. Considine, M.J., MacPherson, E.D., Munro, B.C. (1998) Performance of a branded lamb alliance. *Animal Production in Australia*. 22, 299

Hopkins DL and Thompson JM (2001) The relationship between tenderness, proteolysis, muscle contraction and dissociation of actomyosin. *Meat Science*. *57:1-12*

Hopkins DL, Hegarty RS (2004) Effect of sire type and plane of nutrition on lamb loin shear force, myofibrillar fragmentation index and calcium concentration *Journal of Muscle Foods* 15, 109–120

Hopkins DL, Martin L, Gilmour AR (2004) The impact of homogenizer type and speed on the determination of myofibrillar fragmentation *Meat Science*, *67, 705-710*

Hopkins DL, Hegarty RS, Farrel TC (2005) Relationships between sire estimated breeding values and the meat and eating quality of meat from their progeny grown on two planes of nutrition *Australian Journal of Experimental Agriculture,* 45, 525-533.

Hopkins, D. L. and Thompson, J.M. (2001). Inhibition of protease activity 1. The effect on tenderness and indicators of proteolysis in ovine muscle. *Meat Science,* 59. 175-185.

Hwang, I.H., Devine, C.E. and Hopkins, D.L. (2003). Review: the biochemical and physiological effects of electrical stimulation on beef and sheep tenderness. *Meat Science*. 65. 677 – 691.

Koohmaraie M, Kent MP, Shackelford SD, Veiseth E, Wheeler T (2002) Meat tenderness and muscle growth: is there any relationship. *Meat Science* 62 345-352.

Lee, S., Polidori, P., Kauffman, R.G., and Kim, B.C. (2000). Low-voltage electrical stimulation effects on proteolysis in lamb tenderness. *Journal of Food Science*. **65**. pp 786-790.

McDonagh MB, Ferguson KL,. Bacic A, Gardner GE Hegarty RS (2006) Variation in protein abundance profiles in the M. semitendinosus of lambs bred from sires selected on the basis of growth and muscling potential. *Australian Journal of Agricultural Research,* 57, 671-682

O'Halloran, J.M., Ferguson, D.M., Perry, D., and Egan, A.F. (1998). Mechanisms of tenderness improvement in tenderstretched beef carcasses. In *'Proceedings of the 44th International Congress of Meat Science and Technology'*, Barcelona, Spain. pp 712-713.

Perry D, Shorthose WR, Ferguson DM and Thompson JM (2001) Methods used in the CRC program for the determination of carcass yield and beef quality. *Australian Journal of Experimental Agriculture, 41:953-957.*

Pethick DW, Davidson R, Hopkins DL, Jacob RH, D'Souza DN, Thompson JM Walker PJ (2005a) The effect of dietary treatment on meat quality and on consumer perception of sheep meat eating quality. *Australian Journal of Experimental Agriculture, 45, 517-524.*

Pethick, D.W., Ferguson, D.M., Gardner, G.E., Hocquette, J.F., Thompson, J.M., and Warner R. (2005b). Muscle metabolism in relation to genotypic and environmental influences on consumer defined quality of red meat. In 'Indicators of milk and beef quality' EAAP Pib. No 112. (eds JF Horquette, S Gigle. pp 95-110. Academic Publishers, Wageningen, Netherlands.

Polidori, P, Lee, S, Kauffman, R.G., Marsh B.B (1999). Low voltage electrical stimulation of lamb carcasses; effects on meat quality. *Meat Science*. 53. 179-182.

SAS (1999)

Shaw, F.D., Baud, S.R., Richards, I., Pethick, D.W., Walker, P.J., and Thompson, J.M. (2005) New electrical stimulation methodologies of sheep carcasses. *Australian Journal of Experimental Agriculture.* 45, 575-583.

Shorthose, W.R., Powell, V.H. and Harris, P.V. (1986) Influence of electrical stimulation, cooling rate and ageing on the shear force values of chilled lamb. *Journal of Food Science.* 51, 889-892.

Thompson JM (1990) Correlated responses to selection for growth and leanness in sheep. Proceedings of the Fourth World Congress on Genetics Applied to Livestock Production. Edinburgh, 16:266-275.

Thompson JM (2004) The effects of marbling on flavour and juiciness scores of cooked beef, after adjusting to a constant tenderness. *Australian Journal of Experimental Agriculture* 44, 645 - 652

Thompson JM, Gee A, Hopkins DL, Pethick DW, Baud SR, O'Halloran WJ (2005) Development of a sensory protocol for testing palatability of sheep meat. *Australian Journal of Experimental Agriculture* 45, 469-476.

Thompson, J.M., Hopkins, D.L., D'Souza, D.M., Walker, P.J., Baud, S.R., and Pethick, D.W. (2005) The impact of processing on sensory and objective measures of sheep meat eating quality. *Australian Journal of Experimental Agriculture.* 45, 561-573.

Thomson KL, Gardner GE, Simmons N, Daly C, Thompson JM (2007) Length of exposure to high post-rigor temperatures affects ageing rate of beef. *Australian Journal of Experimental Agriculture.* (submitted)

Tornberg E (1996) Biophysical Aspects of Meat Tenderness. *Meat Science* 43: S175-S191.

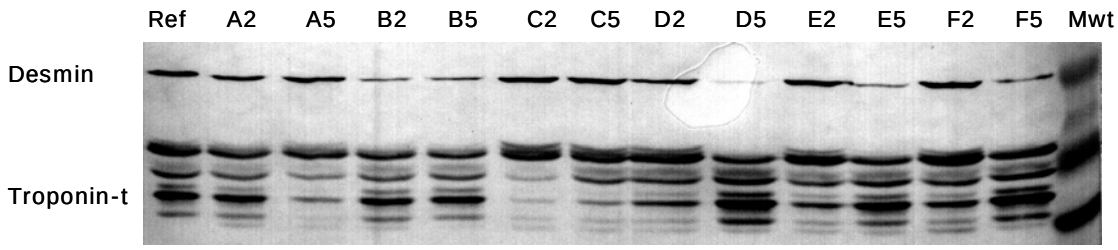

Figure 1: Example of a Western blot gel for muscle proteins desmin and troponin-t. Ref = standard reference sample. Letter refers to the animal, number refers to length of ageing post-mortem (eg. A2 = animal A, day 2 post-mortem). Mwt = molecular weight standard.

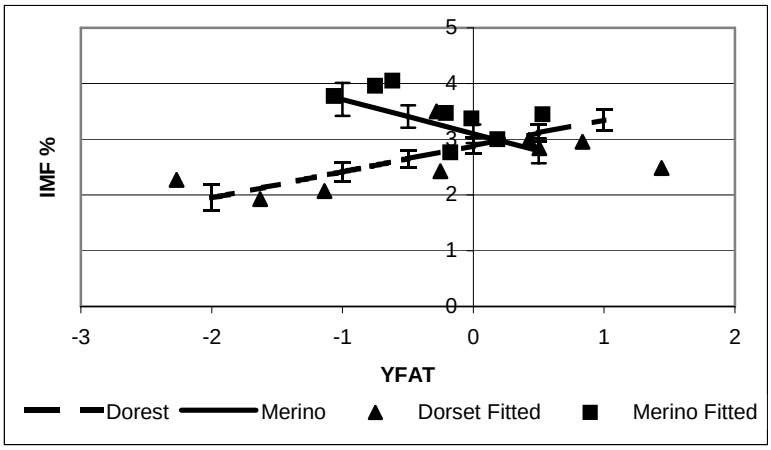

Figure 2: Predicted means for intramuscular fat percentage for the Poll Dorset and Merino sired carcasses at different sire YFAT (mm) ASBVs for experiment 1

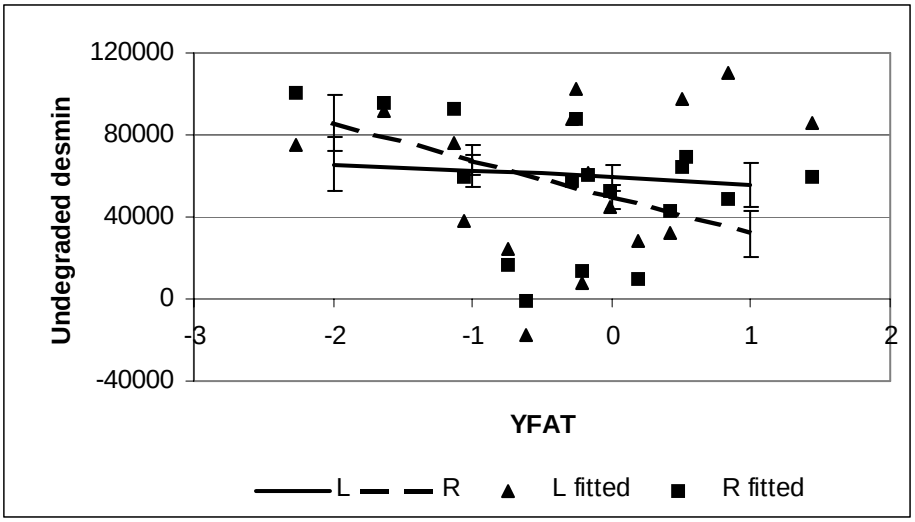

Figure 3: Predicted means for level of native desmin for the left and right sides of the carcass at different YFAT levels for experiment 1.

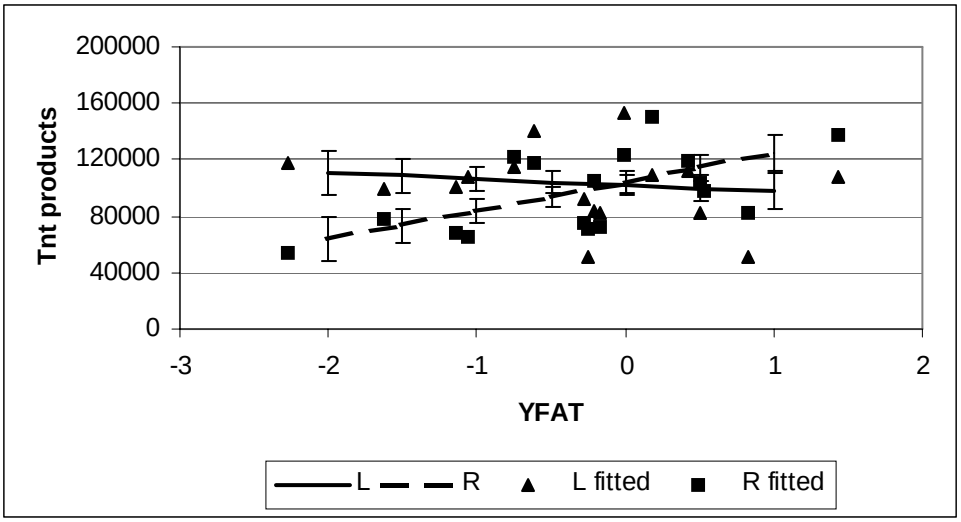

Figure 4: Predicted means for level of troponin-T degradation products (<30kDa) for the left and right sides of the carcass at different YFAT levels for experiment 1

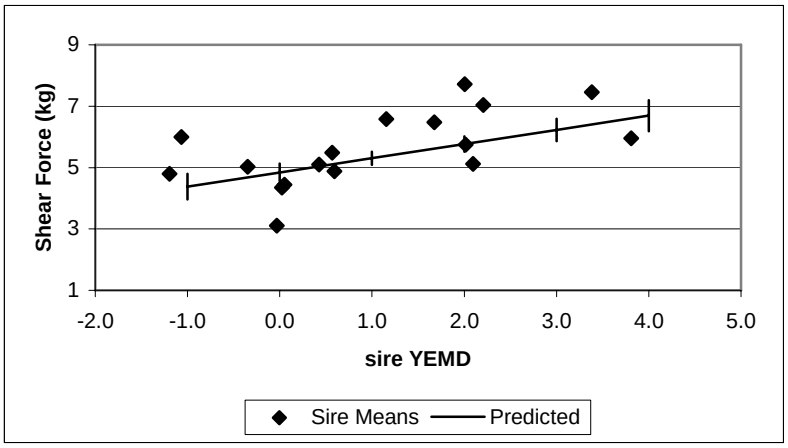

Figure 5: Variation in sire YEMD on shear force (kg) of the SM muscle for experiment 2

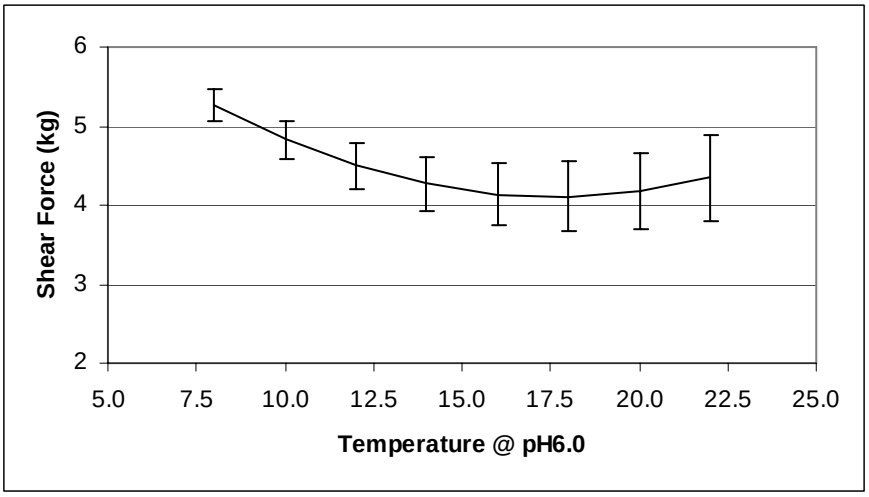

Figure 6: The effect of temperature at pH 6.0 on shear force (kg) in the LL and SM muscles for carcasses in experiment 2.

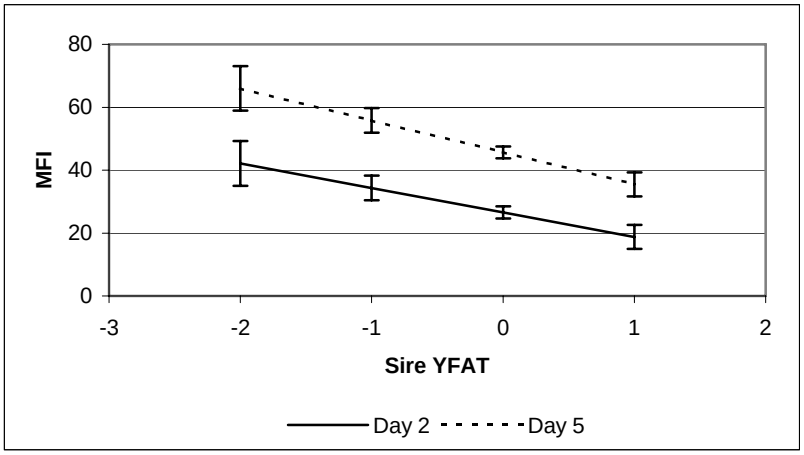

Figure 7: Predicted means for myofibrilar fragmentation index at days 2 and 5 post-mortem at different sire YFAT (mm) ASBVs for experiment 2.

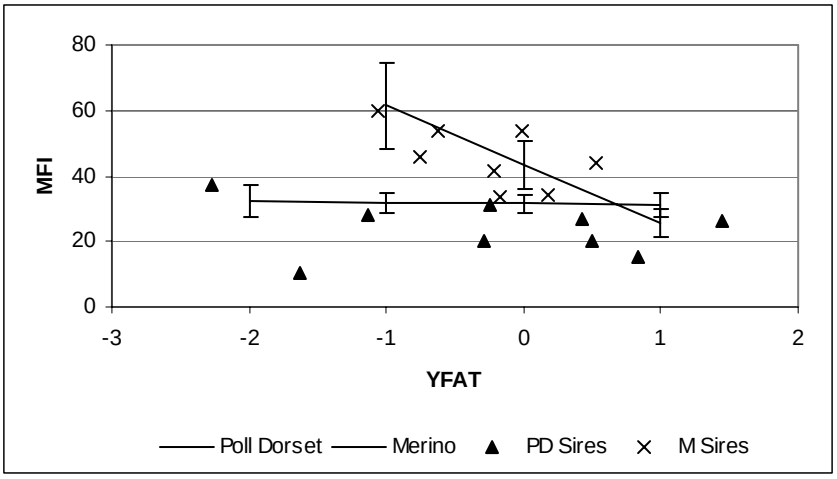

Figure 8: Predicted means for myofibrilar fragmentation index for Poll Dorset and Merino sired progeny at different sire YFAT (mm) ASBVs for experiment 2.

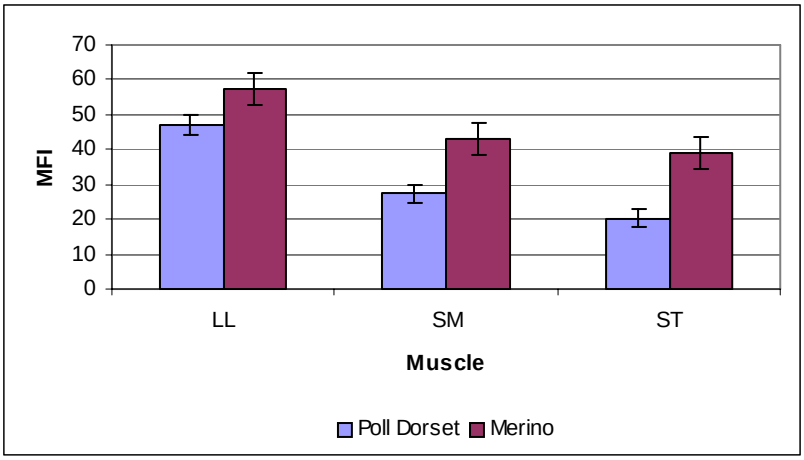

Figure 9: Predicted means for myofibrilar fragmentation index for the LL, SM and ST muscles for both the Poll Dorset and Merino sired progeny in experiment 2.

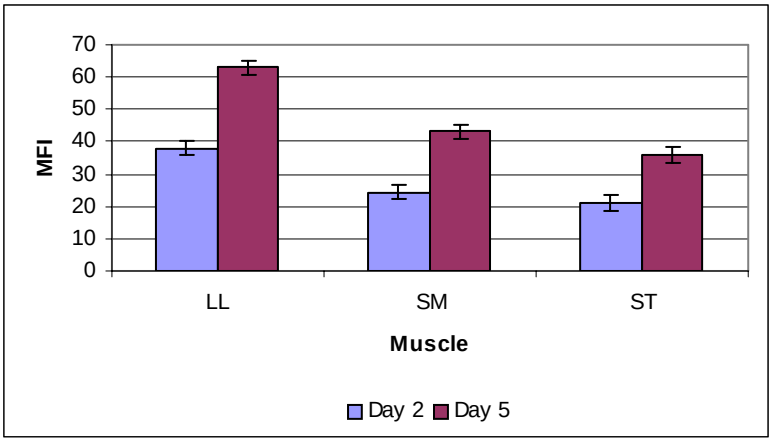

Figure 10: Predicted means for myofibrilar fragmentation index for the LL, SM and ST muscles at day 2 and 5 post-mortem for carcasses from experiment 2.

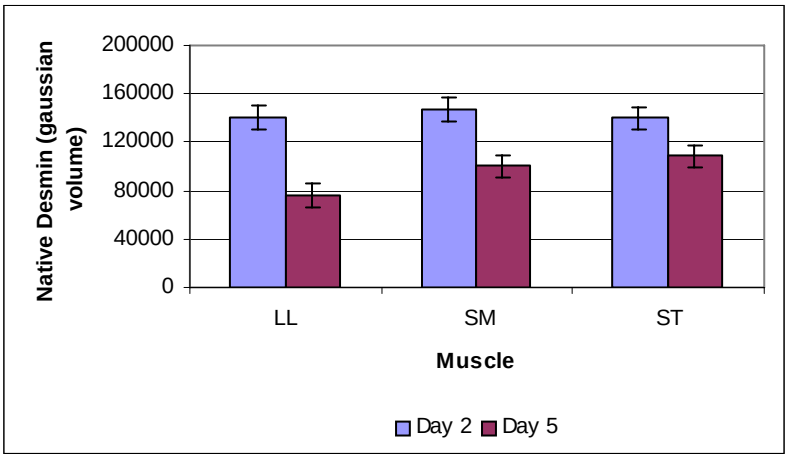

Figure 11: Predicted means for levels of native desmin in the LL, SM and ST muscles at day 2 and 5 post-mortem for carcasses from experiment 2.

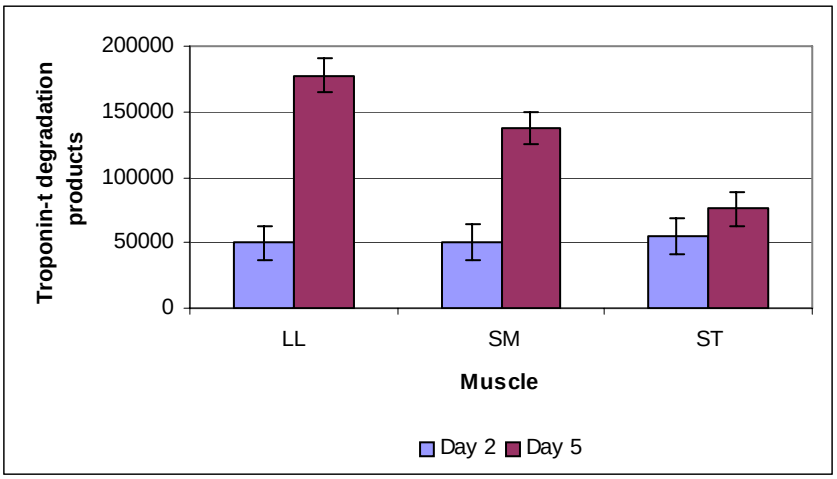

Figure 12: Predicted means for levels of troponin-t degradation products for the LL, SM and ST muscles at day 2 and 5 post-mortem for carcasses from experiment 2.

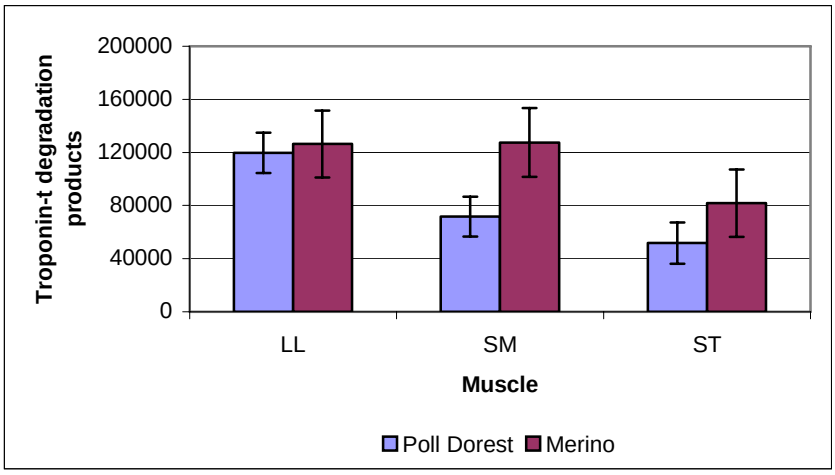

Figure 13: Predicted means for the level of troponin-t degradation products in the LL, SM and ST muscles for both the Poll Dorset and Merino sired progeny for carcasses from experiment 2.

Table 1: Australian Sheep Breeding Values for yearling eye muscle depth (YEMD), yearling growth (YWT) and yearling fat depth (YFAT) for the individual sires used in experiments 1 and 2.

Sire	Sire Breed	YEMD (mm)	YWT (kg)	YFAT (mm)
A	Merino	2.10	8.75	0.53
B	Merino	0.59	-5.15	-0.21
C	Merino	0.57	-0.48	-0.18
D	Merino	0.43	-2.71	-0.62
E	Merino	0.05	9.95	-0.02
F	Merino	0.02	-4.81	-1.07
G	Merino	-0.03	-0.81	0.18
H	Merino	-0.35	-3.55	-0.75
I	Poll Dorset	3.81	3.03	1.44
J	Poll Dorset	3.38	4.70	0.50
K	Poll Dorset	2.21	6.91	0.83
L	Poll Dorset	2.02	7.61	-0.25
M	Poll Dorset	2.01	10.25	-1.63
N	Poll Dorset	1.67	9.04	-1.14
O	Poll Dorset	1.15	2.99	0.43
P	Poll Dorset	-1.07	11.93	-0.29
Q	Poll Dorset	-1.20	6.48	-2.27

Table 2: Mean (± s.d.) and range for carcass and eating quality traits in the *m. longissimus thoracis et lumborum* in experiment 1

Traits	Mean (± s.d.)	Range
Ultimate pH	5.64 ± 0.13	5.33 - 5.95
Temperature at pH 6.0 (ºC)	4.9 ± 0.7	3.6 - 7.0
HSCW (kg)	20.1 ± 4.6	11.8 - 28.6
GR (mm)	17.1 ± 12.2	2.0 – 46.0
Intramuscular Fat %	3.04 ± 0.69	1.73 - 4.61
Sarcomere Length (µm)	1.80 ± 0.15	1.44 - 2.08
Shear Force [A] (Newtons)	68.0 ± 23.5	28.5 – 138.3
MFI [A]	35.4 ± 17.5	4.5 - 70.8
Tenderness score [A]	56.6 ± 13.2	21.6 - 86.8
Juiciness score [A]	55.0 ± 11.0	28.1 - 79.1
Flavour Liking score [A]	59.0 ± 9.7	36.6 - 81.5
Overall Liking score[A]	58.3 ± 10.8	35.7 - 84.6

[A] averaged across both ageing periods.

Table 3: F-ratios (& df) for hot standard carcass weight (kg), GR (tissue depth), Sarcomere length (µm) and Intramuscular fat percentage for experiment 1.

	Hot Standard Carcass Weight (kg)		GR depth (mm)		Sarcomere Length (µ)		Intramuscular fat %	
	NDF, DDF	F-ratio	NDF, DDF	F-ratio	NDF, DDF	F-ratio	NDF, DDF	F-ratio
YEMD	1,58	0.02	1,57	0.41	1,52	0.01	1,51	15.56***
YWT	1,58	4.48*	1,57	3.74	1,52	0.77	1,51	0.84
YFAT	1,58	0.66	1,57	0.04	1,52	0.00	1,51	0.29
Sex	1,58	7.06*	1,57	7.79**	1,52	0.49	1,51	7.14*
HSCW			1,57	39.79***	1,52	0.21	1,51	15.66***
Hang Method			1,57	0.80	1,52	4.55*	1,51	2.49
GR	1,58	43.76***						
Carcass Side					1,52	6.79*		
Sire Breed	1,58	16.44***	1,57	0.02	1,52	4.50*	1,51	0.66
Carwell(sire breed)	1,58	3.30	1,57	0.02	1,52	1.65	1,51	8.63**
YWT x side					1,52	6.36*		
YFAT x Sire Breed							1,51	13.48***

NDF, DDF are numerator and denominator degrees of freedom respectively.
*P <0.05, ** P <0.01; ***P <0.001.

Table 4: F-ratios (& d.f.) for the effect of ASBVs analyses of Shear Force (kg), Intramuscular fat %, Myofibril Fragmentation Index (MFI), Native Desmin (GV) and Troponin-T degradation for Experiment 1. The random term used was animal(sire*sirebreed*sex*hang method).

	Shear Force (N)		MFI		Native Desmin		Tn-T (<31kDa)	
	NDF, DDF	F-ratio	NDF, DDF	F-ratio	NDF, DDF	F-ratio	NDF, DDF	F-ratio
YEMD	1,42	0.84	1,53	11.81**	1,51	3.23	1,52	0.06
YWT	1,42	0.19	1,53	5.81*	1,51	6.02*	1,52	0.01
YFAT	1,42	9.01*	1,53	7.98*	1,51	2.55	1,52	1.14
Sex	1,46	1.88	1,52	0.15	1,48	1.42	1,48	0.06
HSCW	1,42	7.27*	1,53	7.40**	1,51	9.23**	1,52	5.07*
Hang Method	1,46	20.31***	1,52	4.90	1,48	3.31	1,48	6.46*
Carcass Side	1,42	25.7***	1,53	0.01	1,51	3.56	1,52	0.11
Ageing	1,42	82.62***	1,53	103.9***	1,51	81.46***	1,52	132.9***
Sire Breed	1,46	0.27	1,52	0.57	1,48	0.56	1,48	1.55
Pre-cook weight	1,42	4.47*						
Carwell(sire breed)	1,42	0.36	1,53	0.67	1,51	1.07	1,52	2.11
Time of Stimulation	1,42	7.33**	1,53	4.74*				
Desmin Standard					1,51	0.50		
Troponin-T Standard							1,52	3.01
HSCW x Hang Method			1,53	8.47*	1,51	4.92*	1,52	7.52**
Ageing x Hang Method			1,53	5.21*	1,51	4.77*		
YFAT x side					1,51	8.14**	1,52	13.6***
YEMD*side			1,53	6.72*				

NDF, DDF are numerator and denominator degrees of freedom respectively.
*P <0.05, ** P <0.01; ***P <0.001.

Table 5: F-ratios (& d.f.) for the effect of ASBVs for muscling (YEMD), growth (YWT) and fat (YFAT) carcass hang and ageing effects on sensory attributes (Tenderness, Juiciness, Flavour, Overall Liking scores) of grilled loin steaks for Experiment 1. Models included terms for sire breed and carwell nested within sire breed and a random term for animal nested within sire*sirebreed*sex*hang method.

	Tenderness NDF, DDF F-ratio		Juiciness NDF, DDF F-ratio		Flavour NDF, DDF F-ratio		Overall Like NDF, DDF F-ratio	
YEMD	1,63	9.72**	1,63	16.76***	1,63	5.00*	1,63	10.15**
YWT	1,63	1.62	1,63	0.99	1,63	0.01	1,63	0.73
YFAT	1,63	8.39**	1,63	8.45**	1,63	5.67*	1,63	9.47**
Sex	1,58	2.81	1,58	2.43	1,58	1.83	1,58	2.32
HSCW	1,63	2.71	1,63	3.76	1,63	6.70*	1,63	5.15*
Hang Method	1,58	4.53*	1,58	5.52*	1,58	4.22*	1,58	4.55*
Carcass Side	1,63	0.12	1,63	0.06	1,63	1.34	1,63	0.95
Ageing	1,63	13.61***	1,63	9.01**	1,63	15.70***	1,63	11.52**
Sire Breed	1,58	0.02	1,58	0.02	1,58	1.18	1,58	0.49
Carwell(sire breed)	1,63	0.04	1,63	0.16	1,63	0.42	1,63	0.00

NDF, DDF are numerator and denominator degrees of freedom respectively.
*P <0.05, ** P <0.01; ***P <0.001.

Table 6: Predicted Means for the effect of ageing, hang method and regression coefficients for significant ASBVs after adjustment for IMF on sensory attributes (Tenderness, Juiciness, Flavour, Overall Liking scores) in experiment 1.

	Tenderness Mean	s.e	Juiciness Mean	s.e	Flavour Mean	s.e	Overall Like Mean	s.e
Ageing								
Day 2	54.4	1.6	53.3	1.3	57.6	1.2	56.6	1.3
Day 5	59.1	1.6	57.1	1.3	61.6	1.2	60.5	1.3
Hang Method								
AT	53.8	2.0	52.6	1.6	57.5	1.5	56.1	1.6
TS	59.8	2.0	57.8	1.6	61.7	1.5	60.9	1.6
Regression Co-efficients								
YEMD	-3.95**	1.27	-4.06**	0.99	-2.06*	0.92	-3.23**	1.01
YFAT	5.24**	1.81	4.11**	1.41	3.14*	1.32	4.45**	1.45
Regression Co-efficients including IMF								
YEMD	-2.38	1.32	-2.64*	1.07	-1.23	1.01	-2.01	1.07
YFAT	3.52*	1.72	2.54	1.39	2.09	1.31	3.07*	1.39

*P <0.05, ** P <0.01; ***P <0.001.

Table 7: Means (± s.d) and ranges in live weight (kg), hot standard carcass weight (kg) and GR tissue depth (mm) for animals in experiment 2

Trait	Mean (± s.d)	Range
Liveweight (kg)	44.9 ± 7.9	30.3 - 58.7
HSCW (kg)	17.9 ± 3.7	10.6 - 23.4
GR (tissue depth, mm)	5.1 ± 4.3	0.00 – 13.0

Table 8: F-ratios and d.f. for the liveweight (kg), hot standard carcass weight (kg) and GR tissue depth (mm) analysis for animals in experiment 2

	Liveweight		HSCW		Fat (GR)	
	NDF, DDF	F-ratio	NDF, DDF	F-ratio	NDF, DDF	F-ratio
YEMD	1,23	0.06	1,22	1.01	1,22	2.61
YWT	1,23	2.87	1,22	8.14**	1,22	0.08
YFAT	1,23	1.73	1,22	2.79	1,22	0.01
Sex	1,23	0.64	1,22	0.58	1,22	0.20
Sire Breed	1,23	29.01***	1,22	5.04*	1,22	3.94
Carwell	1,23	3.53	1,22	0.31	1,22	0.22
HSCW					1,22	0.58
YWT x Sire Breed			1,22	4.44*		

NDF, DDF are numerator and denominator degrees of freedom respectively.
*P <0.05, ** P <0.01; ***P <0.001.

Table 9: Means (± s.d) for the temperature at pH6.0, sarcomere length, shear force and myofibrillar fragmentation index for both the control and electrically stimulated sides in the LL, SM and ST muscles for animals in experiment 2

Traits		LL			SM		ST	
		N	Mean (± s.d.)	Range	Mean (± s.d.)	Range	Mean (± s.d.)	Range
Temp at pH 6.0	Control	30	6.9 ± 3.8	4.3 – 23.3	8.1 ± 5.4	4.3 – 30.9	9.1 ± 5.8	4.3 – 28.2
	Stimulated	30	7.8 ± 4.0	4.2 – 23.0	8.4 ± 4.3	4.4 – 27.2	9.5 ± 4.1	4.5 – 25.6
Sarcomere Length (µm)	Control	30	1.89 ± 0.16	1.67 - 2.24	1.89 ± 0.15	1.64 – 2.36	2.40 ± 0.24	1.97 – 2.87
	Stimulated	30	1.92 ± 0.14	1.71 - 2.19	1.90 ± 0.16	1.67 – 2.26	2.43 ± 0.20	2.09 – 2.84
Shear Force [A] (kg)	Control	57	6.06 ± 2.31	2.82 – 10.79	6.00 ± 1.52	3.21 – 9.73	-	-
	Stimulated	56	5.26 ± 1.93	3.05 – 12.64	5.46 ± 1.55	3.37 – 9.55	-	-
Myofibril Fragmentation Index [A]	Control	60	47.3 ± 19.7	3.5 – 88.7	28.4 ± 15.9	4.0 – 77.7	21.4 ± 14.2	4.3 – 63.9
	Stimulated	60	46.9 ± 17.2	3.7 – 74.2	29.6 ± 16.8	4.8 – 66.3	25.2 ± 12.6	6.2 – 63.2

[A] Across both ageing periods..

Table 10: F-ratios and d.f. for the effect of sire ASBVs for muscling (YEMD), growth (YWT) and fat (YFAT), electrical stimulation (treatment) and carcass effects on Objective Quality Measures (Sarcomere Length (um), Shear Force (kg) , Cook Loss Percentage (%), Myofibril fragmentation index, Desmin and Troponin-T degradation) for animals in Experiment 2. Models included terms for sire breed and carwell nested within ire breed and a random term for animal nested within sire*sirebreed*sex.

	Sarcomere Length		Shear Force (kg)		Cook Loss %		MFI		Desmin		Troponin-T	
	NDF, DDF	F-ratio	NDF, DDF	F-ratio	NDF, DDF	F-ratio	NDF, DDF	F-ratio	NDF, DDF	F-ratio	NDF, DDF	F-ratio
YEMD	1,161	6.98**	1,178	2.85	1,194	0.30	1,311	2.10	1,282	7.78**	1,289	1.50
YWT	1,161	1.53	1,178	0.56	1,194	0.17	1,311	0.09	1,282	0.52	1,289	1.43
YFAT	1,161	2.89	1,178	0.75	1,194	0.04	1,311	3.04	1,282	1.11	1,289	0.44
Sex	1,161	9.50**	1,23	0.56	1,22	2.55	1,20	0.17	1,22	0.10	1,21	0.41
Sire Breed	1,161	2.39	1,23	0.05	1,22	3.22	1,20	0.01	1,22	3.34	1,21	0.24
Muscle	2,161	275.82***	1,178	1.73	1,194	0.3	2,311	6.08**	2,282	1.80	2,289	8.34**
HSCW	1,161	0.79	1,178	4.09	1,194	1.04	1,311	11.81**	1,282	2.56	1,289	4.02*
Carwell	1,161	1.58	1,178	2.84	1,194	0.11	1,311	1.56	1,282	0.08	1,289	0.09
Position(muscle)	4,161	23.82***	3,178	35.99***	3,194	38.64***	4,311	9.80***	4,282	2.31	4,289	1.50
Treatment	1,161	2.08	1,178	10.25**	1,194	0.44	1,311	7.60**	1,282	0.66	1,289	0.25
Ageing			1,178	64.21***	1,194	24.09***	1,311	361.97***	1,282	158.51***	1,289	182.17***
Desmin Standard									1,282	115.38***		
Troponin-T Standard											1,289	84.17**
Pre-Cook weight			1,178	3.16	1,194	104.58***						
Temp @ pH 6.0			1,178	21.44***	1,194	4.02*			1,282	6.80**		
Temp @ pH 6.0 x Temp @ pH 6.0			1,178	17.74**	1,194	4.43*			1,282	8.38**		
YEMD x muscle			1,178	11.46**								
YFAT x muscle			1,178	7.36**								
YWT x muscle											2,289	6.22**
YEMD x Treatment							1,311	5.90*				
YFAT x Treatment							1,311	4.72*				
YWT x Treatment									1,282	6.01*		
YEMD x Sire Breed									1,282	6.22*		
YWT x Sire Breed							1,311	9.98**			1,289	5.17*
YFAT x Sire Breed							1,311	8.02**				
YFAT x Ageing							1,311	5.54*				
Ageing x Muscle					1,194	10.13**	2,311	8.77**	2,282	6.62**	2,289	28.8***
Sire Breed x Muscle							1,311	5.62**			2,289	3.12*
Sire Breed x Treat									1,282	6.03*		

NDF, DDF are numerator and denominator degrees of freedom respectively.
*P <0.05, ** P <0.01; ***P <0.001.