An investigation into the use of video image analysis (VIA) and visible-near infrared (NIR) spectroscopy for carcase evaluation

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Abstract

In order for the meat industry to move towards a carcase payment system that is more consumer-focused, there is a need to identify carcases that have a higher yield of superior eating quality meat. Through a series of experiments, this thesis investigates the relationships between video image analysis (VIA) variables and saleable meat yield (SMY%) of high-value cuts in beef carcases, and also the relationships between visible-near infrared (NIR) spectra and instrumental meat quality parameters in beef, lamb and venison of various breeds and genders.

Results showed that VIA could effectively replace the visual classifier for classifying beef carcases according to the EUROP carcase classification system, and that both visual and VIA systems showed some promise for predicting the yield of high-value sirloin yield through the EUROP-grid information. Both VIA and visual systems could only account for approximately 57% of the variation in sirloin SMY%, but the relationship between SMY% and other possible VIA outputs such as lengths, widths and volumes remains largely uncharacterized.

Instrumental measures of meat quality (shear force, pH and colour) of *M. longissimus thoracis et lumborum* (LTL) from 234 beef carcases and 208 Texel lambs showed that gender had a larger effect on meat quality than breed. Data from these two experiments was used to determine the relationship between NIR spectra and instrumental meat quality parameters in beef and lamb LTL. NIR showed promise for identifying beef with high ultimate pH values and lamb with high intramuscular fat percentages, but the prediction of shear force using NIR spectra in both beef and lamb was less accurate.

The effects on meat quality of sex, breed, chilled aging and location within venison *M. Longissimus lumborum*, for samples from 79 farmed deer showed that all factors influenced venison meat quality, with aging time and gender having the largest effects. The relationships between NIR spectra and venison meat quality indicated that NIR spectra could be used to identify samples with high ultimate pH and high shear force values.

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The research undertaken in these chapters was part of a larger project looking at the effects of the Texel muscling quantitative trait locus on lamb carcase and meat quality. Financial support of the larger project was from the Biotechnology and Biological Sciences Research Council (BBSRC) (BB/E018963/1) and Defra (LK0670) through LINK (Sustainable Livestock Production Programme), as well as from the Scottish Government. The contributions from industry sponsors and project partners: English Beef and Lamb Executive (EBLEX), Hybu Cig Cymru (Meat Promotion Wales), Quality Meat Scotland, Livestock and Meat Commission Northern Ireland, Pfizer Animal Genetics, AgResearch New Zealand, Innovis Genetic Ltd, Welsh Country Foods Ltd, EplusV GmbH Germany, ASDA stores, Scottish Association of Meat Wholesalers, Suffolk Sire Referencing Scheme Ltd, British Texel Sheep Society Ltd and Charollais Sire Reference Scheme are gratefully acknowledged. Thanks go to SAC farm and technical staff and to technical staff from the Institute of Biological,

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List of abbreviations

Abbreviation	Explanation
AHDB	Agriculture and Horticulture development board
ASD	Analytical Spectral Devices
AU	Australia
AYPG	Adjusted preliminary yield grade
BBSRC	Biotechnology and Biological Sciences Research Council
BCC	Beef classification centre
BF	*M. biceps femoris*
BIOSS	Biomathematics and Statistics Scotland
BON	Bone weight
BONE%	Bone percentage
CAS	Chiller assessment system
CEO	Chief executive officer
CH	Charolais heifer
CL	Cooking loss
CS	Charolais steer
CSL	Complete sirloin
CT	Computer aided tomography
CV	Coefficient of variation
CVS	Computer vision system
DB	Dairy bull
DE	Germany
DFD	Dry, firm and dark
DK	Denmark
DMRI	Danish Meat Research Institute
DO%	Dressing out percentage
DS	Dairy steer
EAAP	European Association of Animal Production
EBLEX	English Beef and Lamb Executive
EC	European Community
EEC	European Economic Community
EJ	Expressed juice
ES	Spain
EU	European Union
F	France
FAT%	Fat percentage
FIL	Fillet weight
GB	Great Britain
GLM	General linear model
HCS	Hot carcase system
HCW	Hot carcase weight
HU	Hungary
IF	Intermuscular fat
IQR	Inter-quartile Range
IYF	Initial yield force
KgF	Kilograms of force

Abbreviation	Explanation
KKCF	Kidney, knob and channel fat
LH	Limousin heifer
LL	*M. longissimus lumborum*
LMCNI	Livestock and Meat Commission Northern Ireland
LMY%	Lean meat yield percentage
LT	*M. longissimus thoracis*
LTL	*M. longissimus thoracis et lumborum*
LS	Limousin steer
MAC	Machine à classer
MANOVA	Multivariate analysis of variance
MARC	Meat Animal Research Centre
MEQ	Meat eating quality
MIRINZ	Meat Research Institute of New Zealand
MLA	Meat and livestock Australia
MLC	Meat and livestock commercial
MSA	Meat standards Australia
MSC	Multiplicative scatter correction
NIR	Near infrared spectroscopy
NO	Norway
NZ	New Zealand
PLSR	Partial least squares regression
QMS	Quality Meat Scotland
REML	Restricted maximum likelihood
RMS	Research Management Systems
RMSE	Root mean square error
RPD	Ratio performance deviation
RSD	Residual standard deviation
SAC	Scottish Agricultural College
SD	Standard deviation
SE	Standard error
SED	Standard error of the difference
SF	Subcutaneous fat
SL	Sarcomere length
SM	*M. semimembranosus*
SMY%	Saleable meat yield
SNV	Standard normal variate
SS	Saleable sirloin
SZ	Switzerland
TM-QTL	Texel muscling quantitative trait locus
UK	United Kingdom
USA	United States of America
USDA	United States department of agriculture
UY	Uruguay
VBM	Value-based marketing
WBSF	Warner-Bratzler shear force
WD	Work done
WHC	Water holding capacity
WTP	Willingness to pay
VHVC	Very high value cuts

Abbreviation	Explanation
VIA	Video image analysis
VL	*M. vastus lateralis*
XSF	Excess fat
YB	Young bull

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1 Introduction

The production, processing and selling of meat can be broken down into a series of transactions that form a value-chain. Farmers breed and finish animals which they sell to meat processors who process the animal into meat and co-products. Processors supply meat products to retailers for sale to consumers. Value is added at each step of the chain and players operating at each step aim to sell the value-added commodity/product for a profit. Within the commercial meat value chain, saleable meat is the most valuable component in the carcase. After processing into cuts, meat is sold based on a weight and specification (such as the type of cut) but the transaction between the producer and processor relies on evaluation of the carcase.

The objective of carcase evaluation is to determine the value per unit weight of the carcase as this should form the basis of the transaction between the producing and processing sectors of the meat industry. Three main constituents of a carcase are lean meat, fat and bone. Where the percentage of lean meat yield (LMY%) in the carcase is determined by the percentage of fat (FAT%) and the ratio of muscle weight to bone weight (Purchas *et al.* 2002b). Similarly, SMY% refers to the weight of lean meat plus a specified amount of fat as a percentage of carcase weight meaning that SMY% is usually greater than LMY% for a given carcase. Because of different market requirements for the amount of fat left on the cut of meat, SMY% tends to be less consistent than LMY%. The most variable carcase component is FAT%; often excess fat must be removed for the product to meet market specifications – adding to processing cost. Accurate, precise predictions of both SMY% and FAT% is therefore of great importance when evaluating carcases in order for the evaluation to reflect the main value component in the carcase - the yield of saleable meat.

Carcase evaluation worldwide involves sorting carcases into categories so a value can be assigned. Grading and classification are terms that are often used interchangeably; both sort carcases into categories but there are some differences. Grading aims to sort carcases based on merit or worth, thus imputing a value element, while classification aims to categorize carcases on the basis of a standard description leaving the purchaser

to determine which category is best suited to their needs (Kirton 1989). Classification is thus often more objective than grading and is more widely used (Kempster *et al.* 1982a).

In the United Kingdom (UK), beef and lamb carcases are currently classified by trained assessors according to the EUROP classification scheme where each letter (E, U, R, O and P) correspond to a conformation class (Fisher 2007). Because conformation includes both fat and lean meat, a second factor (usually a numerical value between 1 and 5) is used alongside the conformation to account for the percentage of fat in the carcase. The combination of a conformation class and fatness factor along with information on the age and gender of the carcase correspond to a point on a pricing grid, which, when multiplied by the carcase weight completes the evaluation process as far as the producer is concerned.

Meat processors add value to the carcase through conditioning (allowing the meat to tenderize), fabricating (preparing saleable cuts of meat) and packing the meat into portions ready for the end user - the consumer. A number of animal production factors such as growth path (Purchas *et al.* 2002a), genotype (Shackelford *et al.* 1995; Maltin *et al.* 2001; Prieto *et al.* 2011) and gender (Purchas and Aungsupakorn 1993; Sinclair *et al.* 1998) can affect meat quality. Arguably, meat processing techniques have even larger effects on meat quality where a number of interventions such as electrical stimulation (Davey *et al.* 1976; Hwang and Thompson 2001; Hwang *et al.* 2003), chilling rate (Aalhus *et al.* 2001) and aging time (Farouk *et al.* 2009) play an important role in ensuring optimal beef meat quality.

Because consumers are the ultimate arbiters of meat eating quality, factoring meat eating quality into carcase evaluation is necessary to ensure that the meat value chain can match production and breeding goals with consumer demand. The total food quality model (Grunert 2005) explains that consumers are much more likely to repeat an initial purchase of meat when the actual eating quality of the meat exceeds the expectations they formed at the point of purchase. Unfortunately information available to the consumer at the point of purchase is usually not indicative of the actual eating quality (taste, texture, juiciness etc.). Consequently, there is a need for additional meat quality indicators (such as an eating quality guarantee) that relate closely to the actual meat eating quality. Consumers may use such information to make more-informed purchase

decisions and such information could also be factored into carcase evaluation systems. This concept is known as value-based marketing (VBM) and has gained much more traction in the beef industry (Cross and Whittaker 1992; Polkinghorne and Thompson 2010). The benefits of such a system to the meat industry are two-fold, firstly, there is extensive research that concludes consumers are willing to pay a premium for superior eating quality and secondly, an increase in repeat purchases can be expected due to consumers being satisfied with their initial purchase (Grunert 2005).

Despite this, there is little emphasis on meat quality in current classification methods used worldwide to evaluate carcases, although there is a quality grade in the USA and Canada and some Asian countries measure the level of intramuscular fat (marbling) in the *longissimus* muscle.

The meat standards Australia (MSA) system is also an exception in that individual cuts are appraised and labelled with appropriate ratings for meat quality (comprising of tenderness, juiciness, flavour and overall liking) with various cooking methods (Polkinghorne and Thompson 2010). Despite the intricate detail of the MSA system, beef carcases are still classified into boning groups based on carcase weight, maturity (dentition) and back fat depth, no premiums for producers can be gained based on objective measures of meat quality. This is mainly because there are very few methods of predicting meat quality that are suitable for abattoir conditions. Various methods have been devised for measuring or predicting meat quality in an intact or quartered carcase although few have been used routinely for carcase evaluation. Until a suitable method of quantifying meat quality is identified, carcases evaluation is unlikely to comprehensively embrace all or most elements of meat quality, consumer preferences will not be accurately reflected in prices paid to the producer.

It is arguable that meat quality may actually be assessed indirectly in systems that differentiate carcases on age, gender and fatness. For example, carcasses of older animals are known to have higher proportions of insoluble collagen resulting in tougher meat (Young and Braggins 1993). Fatter carcases may be less susceptible to the phenomenon of cold shortening due to the insulating effects of fat. A rapid drop in muscle temperature before sufficient pH decline *post mortem* can result in an early onset of rigor and cold shortening which is linked to toughness in meat (Dransfield and

Rhodes 1976). Similarly, many experiments have reported the meat of bulls to be tougher than that of steers (Reagan *et al.* 1971; Purchas and Aungsupakorn 1993; Purchas and Grant 1995; Purchas *et al.* 2002a). The biological mechanisms underpinning these various effects are important, but there is still variation in meat quality characteristics within these groups. In considering this problem through an outcome-based approach, there is a need to develop tools that can be used to improve the consistency of quality meat on offer to the public, irrespective of the biological variation.

Improvement (and refinement) of carcase evaluation based on both SMY% and meat quality parameters requires safe, cost effective, non-destructive and preferably objective measurement or prediction of SMY% and at least some aspects of meat eating quality. Two technologies have been identified that may provide solutions to the problem. Video image analysis (VIA) technology has been developed to predict SMY% in a highly consistent way (Allen and Finnerty 2000). Visible-near infrared (NIR) spectroscopy is able to assess some meat quality parameters (Prieto *et al.* 2009a). Both technologies scan the carcase, the former operates on the intact carcase side whereas the latter scans the meat surface. The accuracy, precision and consistency of VIA and NIR operating under commercial abattoir conditions has not been extensively investigated.

Through a series of experiments, the broad aim of the research reported in this thesis was to investigate the relationships between VIA-predicted variables and the yield of high-value trimmed, boneless cuts in beef carcases and to investigate relationships between NIR spectra and instrumental meat quality parameters in beef, lamb and venison of various genders and genotypes.

The main emphasis for the VIA section is on determining the ability of the technology to measure the SMY% and composition of the high-value loin region. In terms of NIR, the main emphasis is on identifying meat where the quality is poor with a view to improving the consistency of whole meat products on offer to consumers rather than to elucidate the biological mechanism underpinning the relationship between NIR spectra and meat quality *per se*.

1.1 Specific objectives

1. To summarize the development and application of VIA for evaluation of beef carcases and discuss the advantages, shortfalls and future research needs of the technology (Chapter 2).

2. To appraise the performance of NIR spectroscopy for predicting meat quality traits and identify the areas where further research is required (Chapter 2).

3. To compare carcase traits and the trimmed, boneless yield of sirloin and fillet meat for groups of cattle differing in gender and breed and assess the accuracy with which these characteristics were predicted by VIA and visual carcase classification systems (Chapter 3).

4. To characterize the effects of gender and genotype on instrumental measures of beef quality in the *M. longissimus thoracis* and evaluate the effectiveness with which NIR spectroscopy used under abattoir conditions predicted instrumental measures of beef quality in the *M. longissimus thoracis* (Chapter 4).

5. To evaluate sex effects on lamb meat quality parameters of *M. longissimus lumborum* and *M. semimembranosus* in Texel ram and ewes lambs and characterize the effect of the TM-QTL on meat quality parameters of *M. semimembranosus* (Chapter 5).

6. To determine the ability of NIR spectroscopy data collected on fresh (never-frozen) lamb *M. longissimus lumborum* to predict instrumental meat quality parameters of *M. longissimus lumborum*, *M. semimembranosus* and *M. vastus lateralis* (Chapter 6).

7. To investigate the effects of sex, genotype, sampling location and chilled aging on meat quality parameters of venison short-loin produced, processed, and aged under commercial conditions (Chapter 7).

8. To determine whether NIR spectra can be used to predict instrumental meat quality parameters of venison *M. longissimus lumborum* (Chapter 8).

9. To present findings as a summary in a suitable format for meat industry use (Chapter 11).

2 Review of the literature

Publications based on this chapter:

Craigie CR, Navajas EA, Purchas RW, Maltin CA, Bunger L, Hoskin SO, Ross DW, Morris ST, Roehe R (2012) A review of the development and use of video image analysis (VIA) for beef carcase evaluation as an alternative to the current EUROP system and other subjective systems. *Meat Science* **92,** 307-318.

Abstract

The first part of this review discusses carcase evaluation and focuses mainly on beef carcase evaluation. The development and use of video image analysis for beef carcase evaluation is considered as an alternative to the current EUROP system and other subjective systems. Such systems are still largely dependent on visually assessed fatness and conformation; their purpose is to provide a common basis for the description of carcases for use in trade, price reporting and intervention. The meat industry requires a carcase evaluation system based on accurately predicted saleable meat yield (SMY%) and meat eating quality parameters. The current EUROP carcase classification system shows highly variable correlations to SMY% due in part to the variable distribution of fat throughout the carcase as affected by breed, sex, diet, and the level of fat trimming, and has no provision for meat quality. Video image analysis technology is capable of improving the precision and accuracy of SMY% prediction even for specific carcase joints and simultaneously mimics the visual assessment to comply with EU regulations on carcase classification. The second part of this review explains meat eating quality, how it is routinely measured and also discusses why meat eating quality is important for consumers. The third part of the review considers visible-near infrared (NIR) spectroscopy and reviews the ability of NIR in conjunction with chemometric analyses to predict meat quality parameters in beef and lamb in a fast, non-destructive, safe, accurate and repeatable way.

2.1 Introduction

The evaluation of beef, lamb and venison carcases usually forms the basis for the transaction between livestock producers and meat processors. Carcase classes or grades are defined in such a way that they describe carcases on the basis of some selected characteristics that determine merit or value per unit of carcase weight. Frequently, carcase classification or grading is a topic of contention, and hence it is important that the process is fair, accurate (un-biased) and precise (repeatable), and that it is standardized.

The evaluation of beef, lamb and venison carcases is achieved through the use of a systematic method of determining the merit or value of a carcase against a standard description which differs by species and among countries. Polkinghorne and Thompson (2010) reviewed beef carcase evaluation systems employed in different countries. The common element being that carcase value per unit weight is largely determined by the yield of saleable meat (SMY%) and to a lesser extent the eating quality of the meat. In contrast, carcase lean meat yield (LMY%) is determined by the percentage of fat (FAT%) in a carcase and the ratio of muscle weight to bone weight (Purchas *et al.* 2002b). Commercial carcase processing involves a considerable level of dissection but a distinction needs to be made between LMY% and SMY%. The former relates to lean meat (i.e. meat with all visible fat and bone removed) expressed as a percentage of carcase weight and the latter refers to the weight of lean meat plus a specified amount of fat and sometimes bone (depending on the market) as a percentage of carcase weight. As a result, SMY% is usually greater than LMY% for a given carcase, and owing to different market requirements for the amount of fat left on the cuts of meat, SMY% tends to be less consistent than LMY% between different experiments. The fact that some cuts such as a rack or leg of lamb are sold inclusive of bone add further complication to the definition of SMY%.

Almost all carcase evaluation systems reward producers for higher yields of saleable meat and there is usually no direct assessment of meat eating quality. Meat eating quality (MEQ) parameters such as tenderness, juiciness and flavour are subjective and difficult to define measure and predict. As a consequence, the direct measurement or prediction of MEQ at the point of carcase evaluation is not well developed.

Selection for lean meat yield and growth rate has been shown to have an impact on the meat quality, because it changes the proportions of the main muscle fibre types. For example, selecting for very lean pig carcases had profound effects on the muscle fibre composition of the pork, reducing the percentage of slow oxidative fibres and increasing the percentage fast glycolytic fibres which are associated with increased toughness in pork (Maltin *et al.* 2003). Slow oxidative fibres have also been associated with tenderness in lamb (Wojtysiak *et al.* 2010) and the percentage of slow fibres is lower in the *M. longissimus lumborum* (LL) in Texel lambs than in the Scottish Blackface lambs which are, regarding carcase quality, a less-improved breed (Bunger *et al.* 2009). The same trend has also been seen in cattle with highly-muscled Belgian blue cattle having a lower number and percentage area of slow oxidative fibres in their muscles than Galloway, German Angus and Holstein Friesian breeds (Wegner *et al.* 2000). Over a period of time, selection for yield-related traits without consideration for meat eating quality is likely to have a negative impact on tenderness, juiciness and flavour.

In general, the various visual carcase evaluation systems used worldwide correlate with commercially desirable yield traits in the carcase, yet it has been argued that the actual application of such systems is their major shortfall (Hedrick *et al.* 1969; Borggaard *et al.* 1996; Ruiz de Huidobro *et al.* 2004). Moreover, visual carcase classification is inappropriate for the measurement of phenotypes required for performance recording and breed improvement in terms of carcase and MEQ traits due to variability arising from human error (Conington *et al.* 2006). Specifically, it is essential for performance recording that the measured phenotype reflects the true variation in the animal with minimal variation arising from the assessment method or the preparation of the carcase. In response to these issues, there is a drive to move towards a carcase evaluation system based on SMY% and MEQ which can therefore address the needs of industry in terms of product quantity and quality (Cross and Whittaker 1992). Indeed, the quest to objectively evaluate carcase traits that relate to the SMY% and MEQ has been an active area of research since the 1980's (Cross *et al.* 1983). In particular, the focus has been on using video image analysis (VIA) for carcase evaluation and visible-near infrared (NIR) spectroscopy for meat evaluation because both VIA and NIR are non-destructive, non-invasive, objective, cost-effective, safe, and can be automated in the slaughter chain at line speed.

The objectives of this review are to:

- Summarise the development and application of VIA for evaluation of beef carcases and discuss the advantages, shortfalls and future research needs of the technology.

- Appraise the performance of NIR spectroscopy for predicting meat quality traits and identify the areas where further research is required.

2.2 Carcase evaluation

In the United Kingdom (UK), scientific inquiry into carcase composition was underway as early as the 1850's (Lawes and Gilbert 1857). Indeed, the importance of a common carcase description became paramount when the chilled export trade began to grow in the late 19th century (Kempster *et al.* 1982a). Yet it was not until 1928 that beef carcase grading in the UK (largely based on show ring attributes) was proposed and finally materialized when support payments were introduced after the Second World War (1939-45) according to the Agricultural and Horticultural Development Board (AHDB Industry Consulting 2008).

2.2.1 Grading and classification

The terms carcase *classification* and carcase *grading* are often used interchangeably because they refer to similar procedures, yet grading and classification have differing objectives and underlying assumptions, especially in the UK. The definition of carcase classification given by the AHDB (AHDB Industry Consulting, 2008) is: "A common descriptive language that defines – without any cachets of quality – those characteristics of carcases and meat that would be useful in trading". Carcase *grade* implies that carcases are evaluated in terms of merit from the most preferred to the least preferred 'grades' assuming that all buyers in the carcase market have the same preferences with regard to carcase and meat eating quality (Kempster *et al.* 1982a). Important differences between classification and grading are summarised in Table 2.1.

Table 2.1 Important differences between carcase classification and carcase grading

Classification	Grading
Describes carcases	Imputes value to carcases
No provision for meat quality	Best quality implications
Neutral grade names	Grade names are suggestive of quality
More objective	More subjective

Table adapted from Kirton (1989).

2.2.2 Terminology for describing carcases

Conformation, muscularity and fleshiness are descriptors of carcase shape that have similar meanings, but there are some important differences which need to be taken into account when comparing experiments. *Conformation* is defined as the thickness of muscle and fat in relation to skeletal dimensions, whereas *muscularity* refers only to the muscle thickness or volume in relation to skeletal dimensions and *fleshiness* is the thickness of flesh relative to skeletal dimensions, where flesh is muscle plus intermuscular fat (De Boer *et al.* 1974). Inspectors assess different parts of the carcase such as the fore and hind quarters. The problem with using conformation to describe carcase shape is that it makes no distinction between lean meat and fat components, but this can be overcome to some extent by incorporating a fatness score to account for the fat component of conformation.

2.3 Visual carcase assessment

2.3.1 The EUROP grid

The need for a common carcase classification scale arose when member states of the European Community (EC) began operating in the common beef market in 1968 (EEC No. 805/68 (European Community 1968)) and price reporting to the EC became mandatory. The EUROP grid method of carcase classification was developed for use by countries in the EC who were trading in the common beef market. The EUROP grid system is based on visually-assigned scores for conformation and fat classes which are combined to form a categorical description of the carcase according to the defined standards implemented by European Community Regulations (No. 1208/81 (European Community 1981b) and No. 1026/91 (European Community 1991)). The conformation score ranges from S (Superior) – a less commonly used category (used by some countries to describe double-muscled carcases; notably Spain) via E (Excellent) through to P (Poor) and from 1 (low) to 5 (high) for fat class (European Community 2007). The system is based on the use of a common language to describe carcases using characteristics that are commercially significant (Fisher 2007). When the system was adopted for national classifications yet, no attempt was made to relate

the various classes to levels of LMY% because there was no standard definition of lean meat yield (Allen 2003).

The EUROP system is derived from the 'European Association of Animal Production (EAAP) 15 point scale', which further divides each category into low (–), medium (=) and high (+) sub-categories (De Boer *et al.* 1974). This 15 point scheme was originally proposed to allow comparisons between experiments conducted by different research groups and was considered too complicated for commercial application (Fisher 2007). Due to the large variation in cattle across the EU member states, provision is made for the use of subclasses if the 5 point scale provides insufficient resolution. Table 2.2 shows the subdivisions of the 15 point EAAP scale and those used in the UK.

Table 2.2 The 15 point EAAP scale for classification of beef carcases based on conformation and fatness, the EUROP system and those used in the United Kingdom (UK) - derived from Fisher (2007).

Conformation[a]	Poor			→											Excellent
15 Point Scale	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
EUROP	–P	P	P+	–O	O	O+	–R	R	R+	–U	U	U+	–E	E	E+
UK	–P		P+	–O		O+		R		–U		+U		E	
Fatness[a]	Low			→											Excessive
15 Point Scale	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
EUROP	–1	1	1+	–2	2	2+	–3	3	3+	–4	4	4+	–5	5	5+
UK		1			2			3		4L		4H	5L		5H

[a] Note that the terms "Poor", "Excellent", "Low" and "Excessive" are for illustration only, in application the classification scheme uses the EUROP symbols only.

2.3.2 Use of the EUROP classification scheme in the European Union

EU member states can opt for a sub-set of the 15 point EUROP scale to suit their cattle population. For example in the UK and Ireland, there is greater resolution for fatness at the excessive end of the scale with H and L subclasses used. Out of the EU27, 20 member states use the 5 main classes to describe conformation and 13 member states use the five main fat classes, the remaining states use the full 15 point scales for conformation (n = 7) and fatness (n = 14) (personal communication, Andrzej Piekarewicz, European Commission Directorate-General for Agriculture and Rural Development).

2.3.3 Relationship between EUROP and LMY%

Although no attempt was initially made to relate EUROP scores with SMY% for classification, much research has focused on this area in the past few years. Results summarised in Table 2.3 and Table 2.4 show how point changes in the EUROP grid relate to changes in the yield of lean meat (LMY%) in the whole beef carcase as well as in to the yield of high value cuts relative to carcase weight (the differences between LMY% and SMY% are outlined in Section 2.1). The studies indicated that the percentage of variation (R^2 range 55-75%, Table 2.3) in carcase LMY% explained by the EUROP grid was much greater using the entire carcase than using high-value cuts only (R^2 range 28-57%, Table 2.4), which comprise the cube-roll, strip-loin and fillet (Conroy *et al.* 2009; Conroy *et al.* 2010a; Conroy *et al.* 2010b). While these cuts are a small percentage of the carcase LMY%, they account for a large proportion of carcase value so classification should accurately reflect this. Moreover, for high value cuts a single unit increase in conformation and decrease in fat class were associated with relatively small increases in LMY% (maximum 2.0% and 1.50% for conformation and fatness respectively on the 15 point scale but this varies between studies and sexes (Table 2.4). The EUROP system has been adapted for use on sheep carcases with similarly low accuracies (Johansen *et al.* 2006) and deer carcases (Wiklund and Johansson 2011) but there is little research that has linked EUROP to LMY% in deer.

Table 2.3 Regression coefficients from multiple regression equations indicating changes in lean meat yield (ΔLMY) (grams per kilogram of cold carcase weight [CCW]) per single unit increase (↑) in conformation (Conf) or fat class (Fat) on the 15 point EUROP scale.

Reference	Classification method[a]	*n* / category / mean CCW	ΔLMY per unit ↑ Conf (g/kg)[b]	ΔLMY per unit ↑ Fat (g/kg)[b]	R^2 (%)[c]	RSD (g/kg)[d]
Drennan *et al.* (2007)	Visual	134 steers/319	11.7 (0.77)	–7.2 (1.17)	68	-
	Mechanical	134 steers/319	14.1 (0.78)	–7.4 (0.98)	75	-
Drennan *et al.* (2008)	Visual	48 bulls/323	8.9 (1.78)	–11.9 (1.60)	70	16.0
	Visual	37 heifers/268	8.1 (3.17)	–9.7 (2.09)	55	21.7
Conroy *et al.* (2010a)	Mechanical	74 bulls/322	11.9 (0.91)	–11.1(1.77)	74	20.2
Conroy *et al.* (2009)	Mechanical	336 steers/342	11.2 (0.53)	–8.2 (0.63)	63	22.2
Conroy *et al.* (2010b)[e]	Mechanical	115 bulls/332 40 heifers/293 507 steers/333	11.8 (0.40)	–9.6 (0.47)	73	22.3

[a] Classification method refers to carcase assessment by a trained human assessor (visual) or VIA (mechanical).
[b] Standard errors of regression coefficients are given in parenthesis.
[c] Coefficient of determination.
[d] Residual standard deviation.
[e] Coefficients applied across all genders.

Table 2.4 Regression coefficients from multiple regression equations indicating changes in high value cut (cube-roll, strip-loin and fillet) lean meat yield (grams per kilogram of cold carcase weight [CCW]) (ΔHVLMY) per single unit increase (↑) in conformation (Conf) or fat class (Fat) on the 15 point EUROP scale.

Reference	Classification method[a]	*n* / category / mean CCW (kg)	ΔHVLMY per unit ↑ Conf (g/kg)[b]	ΔHVLMY per unit ↑ Fat (g/kg)[b]	R^2 (%)[c]	RSD (g/kg)[d]
Drennan *et al.* (2007)	Visual	134 steers/319	1.5 (0.14)	–0.8 (0.21)	51	-
	Mechanical	134 steers/319	1.9 (0.15)	–0.72 (0.19)	57	-
Drennan *et al.* (2008)	Visual	48 bulls/323	0.04 (0.39)ns[e]	–1.4 (0.35)	29	3.5
	Visual	37 heifers/268	2.0 (0.55)	–0.3 (0.36)ns[e]	34	3.8
Conroy *et al.* (2009)	Mechanical	336 steers/342	0.96 (0.13)	–1.5 (0.16)	28	5.4
Conroy *et al.* (2010a)	Mechanical	74 bulls/322	1.1 (0.21)	–0.9 (0.42)	28	4.8

[a] Classification method refers to carcase assessment by a trained human assessor (visual) or VIA (mechanical).
[b] Standard errors of regression coefficients are given in parenthesis.
[c] R^2 = Coefficient of determination.
[d] RSD = Residual standard deviation.
[e] ns = not significant ($P > 0.05$).

2.3.4 Limitations of carcase conformation

Defining and interpreting "carcase conformation" is difficult, because the objective is to describe a complex shape using an index (Kempster *et al.* 1982a) and because the definition of conformation includes fat (De Boer *et al.* 1974). Several authors have suggested that there is a poor relationship between carcase conformation and LMY% after correcting for breed and carcase weight, but the opposite is true for fat class (Cole *et al.* 1962; Harries *et al.* 1974; Riordan and Mellon 1978; Kempster and Harrington 1980; Colomer-Rocher *et al.* 1986). Harries *et al.* (1974) previously suggested that the poor relationship reported in their study between "carcase conformation" and traits measured by dissection may be because judges are using different (un-measured) traits to define conformation. Another possible reason for the poor relationship is that carcases have excessive fat (Purchas and Wilkin 1995). This highlights two areas of ambiguity in the application of a carcase conformation score as a measure of carcase SMY%. Firstly, how an individual classifier interprets the reference photographs and subsequently applies carcase classification scales, and secondly, whether or not carcase classification really is a useful predictor of SMY%.

In beef cattle, it has been suggested that conformation and fat scores tend to be positively associated with each other and fatness inversely related to LMY% at a constant conformation (Kempster *et al.* 1982b). This is because the muscle-to-bone ratio tends to be greater in carcases with superior conformation (at a constant fatness, the LMY% will be higher). Also, in certain breeds of sheep, especially those with high fat content, it has been noted that fat and conformation may be confounded (i.e. fatness increased as conformation improved) (Jones *et al.* 1999; Navajas *et al.* 2007). Furthermore, as carcase conformation improves, the resulting estimation of LMY% may be subject to bias if carcase shape (conformation) is used to indicate muscle : bone ratio (Purchas *et al.* 2002b; Johnson *et al.* 2005). Similar arguments exist regarding the use of subcutaneous fatness as a measure of total carcase fat content. Carcases differ in the partitioning of fat between the various fat depots (subcutaneous (SF), *inter*muscular (IF), *intra*muscular (IMF) and kidney knob and channel fat [KKCF]) according to breed, sex and plane of nutrition (Kempster *et al.* 1982a; Fisher and Bayntun 1984). SMY% is currently the main component valued in a carcase and determined by the

FAT% and the ratio of muscle-to-bone. Conformation without accurate determination of the FAT% is, by definition, a suboptimal mode of determining the LMY%.

Carcase conformation and fat class are relatively poor predictors of carcase composition even when applied in a consistent manner (Keane *et al.* 2000; Pabiou *et al.* 2011b). The potential for inconsistency of the classifiers is a widely recognised shortcoming of visual classification - although hard evidence for this is scarce. Johansen *et al.* (2006) reported that trained classifiers tended to over score conformation and underscore fat class of lambs compared to EU reference assessors. The influence of contextual information (information that is unrelated to carcase classification such as work load, supplier details, breed, other tasks such as labelling etc.) on classifier performance is not known. But, as an example, a perceived ideal aesthetic appeal increases consistency at the superior end of the scale, whereas at the poorer end, carcases may be classed as poor for a greater number of reasons (excessive fat cover, different fat colour, inferior muscling etc.) (Warriss 2000). A number of investigators have tested the consistency of judges assessing lamb and beef carcases, and generally noted that consistency of assessment was optimized using a 7-point scale, and that better agreement between judges was noted if photographic references were available (Williams 1969; Harries *et al.* 1974). A scale of 1 to 5 is said to be too narrow for adequate discrimination between classes, a scale greater than 10 is too wide for most people to operate successfully (Kempster *et al.* 1982a). Harries *et al.* (1974) recommended that a 7 category scale should be extended to 10 in order to decrease the distances between points to allow for greater precision. In this sense, accuracy refers to the correct application of a categorical scale, and precision relates to the repeatability of this process. Categorization of a continuous variable does not account for variation contained within a category, so carcase evaluation on a continuous scale is preferable. The prediction accuracy of a continuous variable (such as SMY%) is a combination of the level of bias of the predicted value and the precision (repeatability and reproducibility) of the predicted value. Therefore, to increase both accuracy and precision of carcase evaluation, highly repeatable prediction and reference measures such as LMY% or SMY% are needed.

2.3.5 Alternative methods of carcase assessment

Objective methods of carcase evaluation (other than carcase weight) have been widely investigated in order to increase accuracy and precision. For decades, the gold standard has been either the physical dissection of the whole or half carcase into lean meat, fat, and bone components, where LMY% is the objective, or in the use of expert classifiers where the visual determination of carcase conformation and fat class is the objective. Both approaches have drawbacks, but for different reasons. The physical dissection is very time consuming and expensive and an expert classification is challenged by the lack of total consistency between individual classifiers. Physical dissection is very resource intensive and costly, so there have been many attempts to predict the composition of the whole carcase from the dissection of certain primal joints (Lush 1926; Hammond and Appleton 1932; Hankins and Howe 1946; Callow 1962; Johnson and Charles 1981).

2.3.6 Partial dissection and linear measures as predictors of carcase composition

Interestingly, it has been found that certain primal joints, (such as the 9-10-11th rib section (Hankins and Howe 1946) or the fore-shin (Callow 1962), that are easily separated into lean meat, fat and bone under commercial conditions, can be useful predictors of overall carcase composition for beef carcases with minimal impact on the processing of carcases into saleable meat. For example, by processing the 9-10-11th rib section into lean meat, fat and bone components, Hankins & Howe (1946) estimated correlation coefficients (r) between rib primal LMY% and carcase LMY% to be 0.92 for steers, 0.72 for heifers and 0.85 overall. A major drawback of this approach for predicting carcase composition in industry was that the accuracy of prediction is directly affected by the accuracy with which the joints/tissues can be separated and weighed at line speed in a commercial abattoir (Williams *et al.* 1974). Modern approaches to carcase processing that incorporate robots and other automated processes may reduce the variation in primal cutting (Wadie and Khodabandehloo 1995) but carcase break-down will vary according to customer specification which would need to be accounted for.

Navajas *et al.* (2010a) recently used Computed Tomography (CT) to determine the carcase composition of beef cattle and reported accuracies (R^2 values) of 99%, 92% and 95% for the prediction of muscle, fat and bone tissue weights. These estimates indicate that CT can also be used as an accurate reference method for carcase composition of beef. Even though the beef half-carcase needed to be split into primals and transported to the CT scanner, this technique is more cost-effective and more standardized than physical dissection. In a further study, Navajas *et al.* (2010b) used the CT-scanned fore-rib section to predict the total weight of lean meat, fat and bone in the half carcase. The accuracies of prediction (R^2) were 9%, 60% and 52%, for weight of lean meat, fat and bone respectively, after R^2 values were adjusted for the number of explanatory variables in the model. By additionally adjusting for breed and sex, the amount of variation accounted for increased to 85%, 73% and 67% respectively (Navajas *et al.* 2010b).

2.3.7 Image analysis techniques

Besides CT scanning (which at present has only been used on samples or primals of beef carcases), two main types of image analysis have been applied to predict beef carcase composition on large numbers of beef animals or carcases:

(i) Ultrasonic determination of loin muscle and back fat depth, which has been in use since the 1950s on live animals (Houghton and Turlington 1992), and which has been investigated for carcase evaluation purposes (Cross and Whittaker 1992). Ultrasound continues to play an important role in progeny testing of bulls (Kemp *et al.* 2002) and the wider applications of beef cattle research and management (Lambe *et al.* 2010c).

(ii) Video image analysis (VIA) was developed in the USA specifically for objective beef carcase evaluation in the early 1980's (Cross *et al.* 1983). Since the 1980's, VIA has been applied to many different facets of both carcase and meat eating quality evaluation. Examples include (1) to classify carcases into payment categories based on levels of intramuscular fat at the 12/13th rib (Wyle *et al.* 2003; Jackman *et al.* 2009b), (2) to improve consistency of EUROP classification relative to visual appraisal (Borggaard *et al.* 1996; Allen and Finnerty 2000), (3) to estimate LMY% (Swatland 1995; Hopkins 2008; Rius-Vilarrasa *et al.* 2009), (4) to predict tenderness from the meat surface (Wulf *et al.* 1997; Wyle *et al.* 2003), (5) to quantify the amount of intramuscular fat in beef (Albrecht *et al.* 1996), (6) to measure meat colour (Gerrard *et al.* 1996) and

(7) to evaluate water holding capacity (Irie *et al.* 1996). But this part of the review is concerned primarily with VIA applications relating to beef carcase shape and composition rather than to the prediction of beef eating quality. Meat eating quality will be discussed in Section 2.6. and the application of imaged-based technologies for prediction of meat quality will be discussed in Section 2.7.

2.4 Development of VIA for carcase evaluation

A natural starting point was to investigate the ability of VIA image analysis to measure cross sectional area of the *M. longissimus* interface between the 12th and 13th ribs as it was well established that loin muscle area (LMA) and fat depth information were correlated to composition of the loin. The work of Cross *et al.* (1983) in the USA augmented earlier work (Hankins and Howe 1946; Cole *et al.* 1962) by applying image analysis technology to predict composition of the 9-10-11th rib section, and to quantify intramuscular fat content from the cut surface. Results showed that the VIA approach could explain 89% (LMY%) and 86% (FAT%) of the variation in 9-10-11th rib composition. A VIA system that involved scanning the meat surface formed by quartering between ribs 12 and 13 under fluorescent lighting was used to measure gross fat and muscle areas and convert these into areas, percentages or predictions as required (Cross *et al.* 1983; Wassenberg *et al.* 1986).

Knowing the composition of the 9-10-11th rib was found to be of limited value on its own. In an attempt to improve the prediction, Wassenberg *et al.* (1986) assessed the ability of the same camera-based system to predict the primal (round, loin, rib and square cut chuck) lean cut-out (eight and yield of saleable meat) from 115 steers and compared the performance of VIA to a committee of three trained USDA grading experts. In predicting kilograms of lean meat and LMY%, the VIA data showed R^2 values of 96% and 46%, respectively, which were comparable to the committee scores of 94% and 46%. The model included side weight, lean meat area at the 12/13th rib interface, the percentage of fat area and muscle lightness (colour). The VIA data accounted for less variation in total primal fat weight than the USDA grading experts (R^2 = 68% and 76%, respectively) and both methods accounted for less variation in FAT% than primal fat weight (R^2 = 52% and 65% for VIA and USDA grading experts, respectively). The distribution of lean meat within the carcase was not considered in this

analysis as the meat removed from primals was summed and analysed as a total weight of lean meat and LMY%. Nevertheless, the prototype VIA systems demonstrated that the technology held considerable promise as an objective, non-destructive tool for predicting carcase composition under abattoir conditions.

2.4.1 Refinement of VIA

Refinement of VIA made slow progress for almost a decade in the USA as the result of a decision by the industry that VIA on pre-rigor un-ribbed carcases would be preferable, and that measuring more traits was expensive and prone to failure (Cross and Whittaker 1992). Research into VIA for carcase evaluation continued to progress. In Canada, researchers using the VIAscan (Cedar Creek Company, Australia) to scan the 12/13th rib-surface obtained promising results when predicting LMY% on commercial beef carcases prior to further processing into different cuts of beef (Morgan-Jones *et al.* 1992; Tong *et al.* 1999).

Applying VIA to a meat cut after carcase cutting has been tried a number of times (with variable results) on a section of *longissimus thoracis* from the posterior half of the 12th thoracic vertebra. (Shackelford *et al.* 1998; Teira *et al.* 2003; Farrow *et al.* 2009). Shackelford *et al.* (1998) developed a 5-variable prediction equation, that accounted for 89% of the variation in SMY% across the combined experimental and validation datasets but they did not report the accuracy of their VIA system for predicting FAT% or total fat trim (kg). Teira *et al.* (2003) derived equations to predict individual sub-primal yields using a removed steak that could explain 45% of the variation in pistola cut SMY% (including top round, bottom round, eye round, knuckle, rump, cube roll, strip-loin, and fillet as percentages of carcase weight). Farrow *et al.* (2009) could explain 68% of the SMY% variation in 87 steers (coefficient of variation (CV) = 7.3%) from an image of the 12/13th rib interface with more complex equations. These findings are summarised in Table 2.5. The R^2 and RSD values were very low in the results presented by Teira *et al.* (2003), which is probably because there was very little variation (CV = 4.0%) in SMY% in the Nelore steers used in that experiment. In contrast, a higher amount of variation (CV = 9.6%) existed between steers and heifers in the results of Shackelford *et al.* (1998) and a higher R^2 and lower RSD values for the prediction equation were obtained. This shows that comparisons between experiments

on the basis of R^2 and RSD values should be made in light of the type of carcases used for developing prediction equations, as the variation in SMY% will have a direct effect on these criteria of predictive ability.

Table 2.5 Summary of image analysis experiments where saleable meat yield percentage (SMY%) was predicted from images of the 12/13th rib interface of *M. longissimus thoracis* after removal from the carcase.

Reference	*n* / category[a]	HCW[b] (kg)	Trim level (mm)	R^2 (%)[c]	RSD[c]
Wassenberg *et al.* (1986)[d]	115 steer carcases, unknown genotype	329.1	≤ 12.7	46	1.1
Shackelford *et al.* (1998)[e]	66 Piedmontese steers & heifers	295.8	≤ 7.6	88	2.6
Teira *et al.* (2003)[f]	51 Nelore cross steers	274.6	Devoid	45	0.65
Farrow *et al.* (2009)[g]	87 Continental cross steers	355.6	≤ 6.0	68	2.4

[a] *n* = number of carcases, category refers to their gender and genotype.
[b] HCW = Average hot carcase weight.
[c] R^2 = coefficient of determination and RSD = residual standard deviation.
[d] Wassenberg *et al.* used HCW as a predictor.
[e] Shackelford *et al.* did not use HCW as a predictor.
[f] Teira *et al.* predicted pistola SMY% and used pistola weight in prediction equations.
[g] Farrow *et al.* used HCW as a predictor and reported an adjusted R^2 value.

It was suggested, early in development, that 12/13th rib-surface VIA would not be viable as a stand-alone grading tool and was best suited to augmenting visual appraisals (Wassenberg *et al.* 1986), although it was later argued that this conclusion was premature and was a result of the operational limitations of positioning the hand-held VIA properly at line speed (Shackelford *et al.* 1998). In fact, progress has been made by augmenting (rather than replacing) the current grading process with VIA instruments.

2.4.2 Augmenting USDA grading

Several studies have investigated how VIA can supplement the visual USDA carcase grading system for assignment of yield grade, (Belk *et al.* 1998; Cannell *et al.* 1999; Wulf and Page 2000; Cannell *et al.* 2002; Steiner *et al.* 2003; Wyle *et al.* 2003) and quality grade (Wulf and Page 2000; Wyle *et al.* 2003). Some of these integrated approaches have resulted in commercial developments as outlined in Section 2.4.3. Using the computer vision system (CVS) Beefcam® instrument, Cannell *et al.* (2002) found that a model including expert grader estimates of adjusted fat thickness and percentage of kidney, pelvic and heart fat, as well as measured hot carcase weight, and VIA measured rib eye area, was the most accurate for determining percentage of fat trim. Even though 77% of the variation was explained, the VIA-measured rib eye area only reduced the residual standard deviation for carcase fat weight from 1.40 to 1.39 kg.

Table 2.6 summarises results of studies where an augmentation approach using VIA has been applied to traditional grading techniques in the USA using commercially developed instruments. It should be noted that in these studies, only the SMY% was predicted and not the more informative LMY% (Purchas *et al.* 2002b).

Table 2.6 Results from studies that used imaging technologies to predict saleable meat yield percentage (SMY%), cut yield, fat trim percentage, and adjusted preliminary yield grade (APYG).

Image technology	Dependant variable, [trim level]	Mean (SD)	*N*	Model	R^2(%), RSD	Reference
VIAscan CAS (chiller system)	Cut yield% [6.0 mm]	na[f]	493	VIAREA[a], VIAFAT[b], HCW[c]	55, 1.32	Morgan-Jones *et al.* (1995a)
VIAscan CAS (chiller system)	SMY% [≤ 6.4 mm]	70.66 (1.95)	240	VIAREA[a], ADJFAT[d], HCW[c]	72, 1.12	Cannell *et al.* (1999)
CVS BeefCam	SMY% [≤ 6.4 mm]	71.66 (2.15)	296	VIAREA[a], VIAFAT[b], HCW[c]	60, 1.47	Cannell *et al.* (2002)
CVS BeefCam	Fat trim% [≤ 6.4 mm]	Na	296	VIAREA[a], KPH[e] (expert), ADJFAT[d], HCW[c]	77, 1.39	Cannell *et al.* (2002)
CVS BeefCam	SMY% [≤ 6.4 mm]	Na	290	Yield grade (0.1), KPH[e] (%)	63, 1.20	Steiner *et al.* (2003)
CVS BeefCam	APYG	3.3 (0.5)	290	VIAREA[a], KPH[e] (kg), HCW [c]	89, 0.31	Steiner *et al.* (2003)
Viascan CAS	APYG	3.3 (0.5)	290	VIAREA[a], KPH[e] (kg), HCW[c]	81, 0.40	Steiner *et al.* (2003)
MARC (VBG 2000)	APYG	3.30 (0.62)	800	5 VIA variables (plus HCW[c])	88, 0.21	Shackelford *et al.* (2003)

[a] Video image analysis rib eye area.
[b] Video image analysis fat depth at 12/13th rib.
[c] Hot carcase weight.
[d] Expert grader adjusted fat depth.
[e] Kidney, pelvic and heart fat estimated by expert grader (expert), actual recorded weight (kg) or percentage (%).
[f] na = not available.

It is also important to note that rib-surface VIA systems do not always offer large improvements in accuracy over visual grading. For example in one study, the maximum R^2 for cut yield was 50% (RSD = 1.40) with 3 grader-assessed variables in the model, whereas the accuracy using VIA was R^2 = 55% (RSD = 1.32) with 6 variables in the model (Morgan-Jones *et al.* 1995a; Morgan-Jones *et al.* 1995b; Cannell *et al.* 2002). By observing the principle of maximum parsimony and the fact that the researchers did not report the significance of the added terms, the importance of this difference is questionable. Similarly, in a different experiment, expert graders were able to predict wholesale cut yield with an R^2 of 67% (RSD = 1.33) whereas the best VIA equation, showed an R^2 of 60% (RSD = 1.47) (Cannell *et al.* 2002).

2.4.3 Commercial VIA on the quartered carcase

Based on the results from the use of VIA on the cut surface at quartering, three commercially available grading systems have been developed to measure a variety of

traits. The Chiller Assessment System (CAS) developed by VIAscan in Australia was the first commercial version of this technology (Ferguson *et al.* 1995b). The second system is the CVS Beefcam®, which is a handheld device that was developed by Colorado State University together with Hunter Associates Laboratory, Inc. [Reston, VA] (Belk *et al.* 2000). The third system is the VBG 2000 developed by E+V GmbH in Germany and the US Meat Animal Research Centre (MARC) (Shackelford *et al.* 2003) for use in the USA (http://www.eplusv.de/VBG). There are at least 20 installations of the VBG 2000 in the US and another three in Canada (Furber 2010). All systems have been developed for use at the 12/13th rib interface which is the site of quartering in North America.

The main difference between the three systems in terms of functionality may be due to prediction equations used in the conversion of raw data into estimates of LMY% or SMY%. A system developed on an animal population of a particular breed/breed type or produced under specific production conditions may not adequately explain variation observed in a different population. This is probably why the VIAscan CAS (Australia) and the CVS Beefcam® (USA) produced slightly different results on the same animals, in terms of sub-primal cuts as a percentage of chilled side weight (R^2 = 60%, RSD = 1.3% and R^2 = 63%, RSD = 1.2%, respectively) (Steiner *et al.* 2003). Differences in thresholds used for determining tissue boundaries could explain why the LMA measured by the CVS was ~6% greater than that by the VIAscan. Interestingly the LMA measured by a panel of 4-6 expert USDA graders using a grid pattern approach was between that of the VIA measures, and the prediction accuracy of the panel for determining sub-primal cut yield was appreciably better (R^2 = 71%, RSD = 1.1%) (Steiner *et al.* 2003). Such variation may present a problem if the definition of LMA, LMY% or SMY% is inconsistent across different carcase supply regions. Further complications can be encountered where different trimming specifications are used or where the objective is to predict SMY% rather than LMY%. All of these commercial realities highlight the critical need for a calibration phase when using the VIA system outside of its development conditions. Since different markets prefer half carcases to be quartered at different anatomical locations along the spine, investigation of the predictive ability of measures taken at alternative positions may also be important.

2.4.4 Applying VIA at other quartering points

Worldwide, the quartering locations for carcases tend to vary (Scotland at the 10/11th rib, for example) and in some countries beef sides are de-boned without quartering either before or after chilling. Vote *et al.* (2009) applied the CVS Beefcam® to the 10/11th interface and found that the existing software could not accurately measure fat depths due to the variability in presence, size, and shape of the trapezius muscle. In another example, an experiment involving 73 Japanese black steers, Karnuah *et al.* (2001) found that information recorded with VIA at the 6/7th rib surface could explain 57% of the observed variation in LMY%, 66% in FAT% and 42% in BONE%. There was very little variation in LMY% in the Japanese black steers used in this experiment, with coefficients of variation (CV%) for LMY% of 4.4-4.9%, FAT% of 8.2-13.4% and bone percentage of 9.8-10.2% for the two subsets of steers. LMA can be measured at the 8/9th rib (Smekal *et al.* 2005) and the 11/12th rib (Bozkurt *et al.* 2008) but neither experiment used LMA to predict whole carcase LMY% or SMY%.

The advantages of augmenting other carcase classification systems (such as the current EUROP classification system) with any commercially available hand-held VIA device at the quartering point have yet to be demonstrated. To verify and calibrate the existing commercial rib surface VIA systems at different quartering positions or on carcases with different trim specifications would require software changes and a comprehensive validation experiment. Interestingly, recent research involving VIA technology applied to the meat surface seems to have focused more on predicting meat eating quality rather than LMY% (Tan 2004; Zheng *et al.* 2008; Jackman *et al.* 2009a; Jackman *et al.* 2010).

2.4.5 Whole-side VIA

There have been a number of drivers for the development of whole-side VIA as an alternative approach to the rib-surface systems. In Europe for example, there was a need to mimic the visual classification because a standardized carcase classification system was required by the EU market for trade, price reporting and intervention (AHDB Industry Consulting 2008). This effectively meant that VIA for assessing carcase sides of beef was the preferred approach. Furthermore, predicting carcase composition from the 12/13th rib cross section was not a viable option in markets where quartering is

performed at different positions in the carcase or where carcases are hot-boned. In most cases, is also preferable to evaluate the carcase shortly after slaughter so that suppliers can be paid sooner. Initially, linear dimensions (length, width, depth etc.) of beef carcases were found to have low correlations with carcase composition (Kempster *et al.* 1982a), but ratios between measures of length and width of muscles such as the *M. semimembranosus* and *M. longissimus thoracis et lumborum* vary between carcase classes (Bass *et al.* 1981). The feasibility of measuring a large number of anatomical features on the beef side to search for useful indicators of carcase composition was, like the rib surface VIA, dependant on the potential of automation. Therefore, image analysis was the method of choice. It is simpler to acquire digital images of carcases as they move past a fixed camera than it is to manually position a hand-held camera on the rib surface of a carcase, thus making whole side VIA an attractive alternative to the rib surface VIA systems. From the late 1980's researchers in several countries including, France, Denmark, Germany and Australia focused their efforts on whole-side VIA systems, while industry in the USA pursued an ultrasonic solution to carcase grading (Cross and Whittaker 1992).

2.4.6 Whole-side VIA operation

The philosophy behind the whole-side approach is that it can be integrated into the slaughter chain and work autonomously. Whole-side VIA systems are designed to operate on hot (pre-rigor) intact sides or whole carcases that are suspended by the Achilles tendons (Sørensen 1984; Sørensen *et al.* 1988; Ferguson *et al.* 1995b; Augustini *et al.* 1997). Most systems are fully automated and operate on-line via a handling mechanism that momentarily presents the carcase or side to a camera while the line pauses before returning to the default position allowing the carcase to progress towards the chiller (Borggaard *et al.* 1996). The position of the VIA system is usually close to the chiller so all necessary dressing of the carcase is completed before weighing and evaluation. VIA information is captured, processed, and stored in abattoir records. It is generally accepted that whole-side VIA systems can operate at speeds required by most European beef processing plants. Five whole-side VIA systems are commercially available for beef grading as of June 2012 (Table 2.7) but there are currently (as of May 2012) no CVS systems in operation anywhere in the world (personal communication, Bob Richmond, Research Management Systems Inc. Colorado). All systems operate on-

line and all use similar methods for classifying carcases or predicting SMY%. A feasibility study investigating the ability of whole-side VIA to objectivity classify beef carcases was carried out in Denmark in the early 1980's (Sørensen 1984). Sørensen *et al.* (1988) described the first commercial trial of the original beef classification centre (BCC-1) device developed by the Danish Meat Research Institute (DMRI). The system used a monochrome camera to evaluate either a half or a whole carcase in a stainless steel enclosure, together with an optical reflectance probe to determine fat and muscle depth. Results with 389 fully-dissected carcases showed that similar information about carcase composition to that obtained from the EUROP grid could be obtained objectively with a high repeatability for LMY% (r = 0.94) and similar accuracies, with the BCC-1 explaining 73%, 77% and 79% of the variation in LMY%, FAT% and BONE% respectively. In comparison, visually assessed EUROP conformation (15-point scale) and fatness (5-point scale) could explain 74% 75% and 77% of the variation in LMY%, FAT% and BONE% respectively (Sørensen *et al.* 1988).

Table 2.7 Commercial whole-side video image analysis (VIA) systems, their key outputs, speed of operation (carcases/h), the number and location (countries) of installations.

VIA system	Manufacturer	Key outputs	Speed (*n*/h)	Data collection method	HCW[a]	*n*[b] (country[c])
VBS 2000	E+V GmbH, Germany	EUROP (15-point and regional variations) SMY% Primal weight	120-450	2 images two-dimensional image for carcase outline, structured light (banding) for three-dimensional image, momentary pause while carcase is presented for analysis (non-stop version also available).	YES	> 40 (DE, F, NO, UK, IRE, HU UY)
VIAscan	Cedar Creek Company Australia	EUROP (15-point), carcase and primal LMY (weight and yield). Images from both sides of each carcase	> 1200	No contact with carcase, no stopping required for analysis two-dimensional images only.	YES	na (AU, NZ, UY)
BCC-2	Carometic A/S Denmark	EUROP (15-point)	80-100	Three images; a two-dimensional image for carcase outline corrected for ambient light, structured light (banding) for three-dimensional image, momentary pause while carcase is presented for analysis.	YES	14 (D, DK, F, ES)
Normaclass MAC 2 (being replaced by MAC S)	Normaclass France	EUROP (15-point) scale for conformation and fat, SMY% (when calibrated)	≤ 120	six-camera system, carcase presented by a handling mechanism to cameras (two-dimensional only).	NO	32 (F, SZ)
CVS	RMS Boulder, Colorado	SMY%	300-450	Non-contact and non-stop only two-dimensional images captured.	YES	-

[a] YES/NO indicates whether hot carcase weight (HCW) is used in the prediction equations by each system.
[b] *n* = Number of operating installations, Location = countries where one or more systems are in use, na = not available.
[c] AU = Australia, SZ = Switzerland, D = Germany, DK = Denmark, ES = Spain, F = France, UK = United Kingdom, HU = Hungary. IRE = Ireland, NZ = New Zealand, NO = Norway, UY = Uruguay.

Almost half of the samples were young bulls or male calves and only 17 were steers or older bulls. In Denmark, there was a need to score fat colour because meat tends to darken and fat becomes increasingly yellow with increasing age of cull dairy cows (Shemeis *et al.* 1994). Subcutaneous fat colour was scored on a 10 point scale. This led to the development of the BCC-2, a system that predicts fat colour on the 10 point scale with an R^2 of 89% (S.E.P = 0.59) (Borggaard *et al.* 1996; Madsen *et al.* 1996).

The French company Normaclass developed a VIA system that was first installed in 1993 (personal communication, C. Précetti CEO, Normaclass 2009). Meat and Livestock Australia (MLA) had installations of their VIAscan hot carcase system (HCS) in several plants undergoing commercial testing over the period between 1993 and 1995 (Eldridge 1994; Ferguson *et al.* 1995b). At around the same time, E+V GmbH in Germany developed the VBS 2000, which was first mentioned in the literature in 1997 (Augustini *et al.* 1997). The VBS 2000 consists of a handling unit that presents the side of beef to a camera. Two images are taken, the first is a two-dimensional image, and the second image is a pseudo three-dimensional using structured light (Figure 2.1). The first commercial installation was in 1998 and there are currently around 40 installations of the VBS 2000 (Brinkmann and Eger 2008) located in Germany, France, Norway, Uruguay, UK (Northern Ireland/Scotland), Hungary and Ireland (personal communication, A. Hinz, E+V GmbH).

Figure 2.1 Two-dimensional (left) and pseudo three-dimensional (right) images from the VBS 2000 (Image courtesy of E+V GmbH, Germany).

2.4.7 Overall summary analysis of whole-side VIA

The accuracy of commercial whole-side VIA systems has been investigated a number of times but an overall assessment of accuracy is lacking. The results from publications that included predictions of SMY%, FAT% and BONE% (derived mostly from commercial cutting trials) and visual EUROP conformation and fatness classifications (15 point scales) were subjected to a summary analysis (Table 2.8). The accuracy in each study was derived from average or median published R^2 and RSD values for each analysis presented in the references. The average number of carcases used in analyses is presented for each system. The average and median of the R^2 values from all systems are presented to give an indication of the overall accuracy of commercially available whole-side VIA technology.

It is difficult to draw conclusions on the overall accuracy of VIA in the absence of a clear SMY% definition or the level of trim (which is the case for most of the experiments listed in Table 2.8). The accuracy of SMY% prediction increases as the level of fat left on the carcase decreases (Cannell *et al.* 1999; Vote *et al.* 2009) because fat trimming is a known source of variation. The lower average amount of variation in SMY% explained by the VIAscan system is largely a result of lower adjusted R^2 values reported by Ferguson *et al.* (1995a) based on four small groups of animals (~30) finished on vastly different diets. The poor VIA performance is most likely because there was very little variation in SMY% in the carcases with the coefficient of variation (CV) ranging from 1.7 to 2.7%. This also reduces the average accuracy of VIA technology to predict SMY% when accuracy is expressed as an R^2 value. Excluding these experiments plus the CVS hot carcase system (which operates as a dual component system with the rib-surface camera), on average, VIA accounted for approximately 71% of the variation in SMY% with an average RSD of 1.06 percentage points.

Table 2.8 Average coefficients of determination (R^2) and residual standard deviations (RSD) indicating the accuracy with which whole-side video image analysis systems estimated carcase saleable meat yield (SMY%), carcase fat or carcase bone, and a summary of all results combined.

VIA system/results[a]	SMY (%)	Fat (%)	Bone (%)	Conformation[b]	Fatness[b]	References and notes
BCC-1						
R^2(%) (RSD)	75 (1.23)	86 (1.04)	82 (1.06)	90	63	Madsen & Thodberg (1994)
Number of analyses	1	1	1	1	1	
Average number of carcases in trials	230	230	230	2810	2810	
BCC-2						
Average R^2(%) (RSD)	70 (1.20)	66 (-)	82(-)	90 (0.70)	82 (1.14)	Madsen *et al.* (1996)
Number of analyses	2	1	1	2	2	Borggaard *et al.* (1996)
Average number of carcases in trials	305	476	476	3220	3220	Allen & Finnerty (2000)
VBS 2000						
Average R^2(%) (RSD)	76 (1.58)	75 (1.84)	82 (0.83)	90 (0.76)	83 (1.15)	Augustini *et al.* (1997), Branscheid *et al.* (1999)
Number of analyses	4	3	3	3	3	Allen & Finnerty (2000), Pabiou *et al.* (2011b)
Average number of carcases in trials	196	217	217	1129	1129	
VIAscan						
Average R^2(%) (RSD)	49 (1.24)	80 (1.60)	61 (1.10)	83 (0.80)	85 (1.38)	Ferguson *et al.* (1995a) (10-12mm trim), Morgan-Jones *et al.* (1995a; 1995b)
Number of analyses	13	1	1	1	1	Allen & Finnerty (2000), Vote *et al.* (2009) (3 levels of fat trimming)
Average number of carcases in trials	141	288	288	2969	2969	
VIAscan + CVS Beefcam[c]						
Average R^2(%) (RSD)	66 (1.40)	84 (1.40)	63 (1.10)	-	-	
Number of analyses	1	1	1	-	-	Vote *et al.* (2009) 3 levels of fat trimming
Average number of carcases in trials	288	288	288	-	-	
VIAscan +CAS[d]						
Average R^2(%) (RSD)	71 (0.71)	-	-	-	-	
Number of analyses	2	-	-	-	-	Morgan-Jones *et al.* (1995a; 1995b) (10-12mm trim)
Average number of carcases in trials	465	-	-	-	-	
Summary[e]						
Average R^2(%)	67	78	74	88	78	
Median R^2(%)	70	80	82	90	83	
Total count of analyses	23	7	7	7	7	

Notes: Average coefficient of determination (R^2) and residual standard deviation (RSD) values for each VIA system calculated from a number of published analyses.

[a] Number of analysis = the number of experiments where trait has been investigated.

[b] EUROP conformation and fatness are on the 15 point scale.

[c] The CVS Beefcam was applied at the 10/11th rib (Vote *et al.* 2009).

[d] CAS = VIAscan chiller assessment system.

[e] Summary information is an average and median R^2 of all VIA systems and the total count of analyses is the number of experiments that have investigated the ability of VIA to predict each trait.

2.4.8 Validation of VIA for use in the European Union

For testing any alternative carcase evaluation system, EU guidelines require a concordance trial consisting of a panel of five trained assessors for the comparison based on carcase classification (European Community 2003). The visual assessment system against which VIA is benchmarked in the EU is problematic because the comparison is not representative of "normal" operating conditions, as usually only one trained assessor is classifying carcases at a given time in each processing plant. It would be more appropriate to validate VIA based on its ability to determine carcase composition which could then be related back to conformation and fatness for the purposes of price reporting. Highly accurate, non-destructive methods of determining beef carcase composition using a CT scanner have recently been developed which would be a suitable alternative to dissection of carcases into lean meat, fat, and bone components (Navajas *et al.* 2010a). Undoubtedly there are other factors that must be taken into account for validation and routine on-site auditing of carcase evaluation. Mobile CT scanners are operating in the UK for on-farm determination of sheep carcase composition for breeding purposes (Bunger *et al.* 2011) so it is possible to CT-scan on-site. As an alternative to LMY%, SMY% is a useful reference for VIA under commercial conditions, but it is highly plant-specific rendering it an unsuitable reference measure across different plants unless tight specifications are set.

Despite being an unrealistic mode of VIA validation in the EU, concordance trials are the method used to validate VIA for use in EU member states. Points are awarded (or deducted) for the percentage of carcases that are classified within 0, 1, 2, 3, or greater than 3 subclasses of the median panel reference score on the EUROP 15 point scale (European Community 2003). The same approach has been used to compare VIA and visual classification for research purposes (Branscheid *et al.* 1999; Allen and Finnerty 2000). Results of trials undertaken in the EU with four whole-side VIA systems (Table 2.9) showed less agreement between each VIA system (where data were available) and reference panels on exact fatness compared to exact conformation. Overall, the concordance classes of exact prediction or prediction within ±1, and ±2 classes were 55%, 68% and 92% for conformation and 34%, 47% and 83% for fatness, respectively (Table 2.9).

Table 2.9 The concordance between video image analysis (VIA) systems and panels of classifiers for determining the EUROP conformation and fatness classes on 15 point scales[a].

	Conformation					Fatness				
VIA system/result	Exact	±1pt	±2pts	−1pt[b]	+1pt[b]	Exact	±1pt	±2pts	−1pt[b]	+1pt[b]
BCC-2										
Average concordance (%)	58	-	-	23	16	35	-	-	23	22
Number of analyses	1	-	-	1	1	1	-	-	1	1
Average number of carcases in trials	2226	-	-	2226	2226	2226	-	-	2226	2226
Normaclass MAC-S[c]										
Average concordance (%)	60	97	99	-	-	-	-	-	-	-
Number of analyses	6	6	6	-	-	-	-	-	-	-
Average number of carcases in trials	3159	3159	3159	-	-	-	-	-	-	-
VBS 2000										
Average concordance (%)	54	39	86	14	29	37	47	83	17	27
Number of analyses	8	7	7	1	1	8	7	7	1	1
Average number of carcases in trials	449	195	195	2226	2226	449	195	195	2226	2226
VIAscan										
Average concordance (%)	49	-	-	22	23	30	-	-	32	14
Number of analyses	1	-	-	1	1	1	-	-	1	1
Average number of carcases in trials	2226	-	-	2226	2226	2226	-	-	2226	2226
Total average concordance (%)	55	68	92	20	23	34	47	83	24	21
Total median concordance (%)	56	68	92	22	23	35	47	83	23	22
Total count of analyses	16	13	13	3	3	10	7	7	3	3

[a] Concordance with a panel of classifiers is determined as the percentage that are exact prediction or the percentages of prediction that are within 1 or 2 classes, which is a form of assessment used in the EU to validate VIA systems.
[b] The plus and minus one sub-class deviation were analysed separately by Allen and Finnerty (2001).
[c] Data for the MAC-S was collected by the Institut de l'Elevage (French Livestock Institute) and made available for this analysis courtesy of Cyrille Précetti, Normaclass.

The UK validation for the VBS 2000 was performed in 2010 using carcases from 885 steers, 439 heifers, 511 young bulls and 240 cows (personal communication, Mike Tempest, Livestock and Meat Commission for Northern Ireland [LMCNI]). The VBS 2000 satisfied the requirements in terms of accuracy and precision outlined in EU legislation (European Community 2003) and is now approved for use in the UK. Reports for industry presented the difference between the total panel score and the predicted VIA in terms of the percentage of carcases classified into each conformation and fat score category. Table 2.10 details these results by category. Results presented in this way do not show the concordance between VIA and the reference panel on a carcase level, and should be considered in light of the distribution of carcases across fat and conformation classes as the sample size will be much smaller at the extremes of the scales.

Table 2.10 Percentage of each category of animal assigned to each carcase conformation and fat class by the 5-person panel and by the VBS 2000 video image analysis system (personal communication, Mike Tempest, LMCNI).

Category	steer (*n* = 885)			heifer (*n* = 439)			young bull (*n* = 511)			cow (*n* = 240)		
	Panel (%)	VIA (%)	Diff[c]	Panel (%)	VIA (%)	Diff[c]	Panel (%)	VIA (%)	Diff[c]	Panel (%)	VIA (%)	Diff[c]
Conf[a]												
E	-	-	0	-	-	0	4	1	–3	-	-	0
U	13	9	–4	15	9	–6	29	19	–10	4	-	–4
R	29	34	+5	51	55	+4	30	40	+10	13	17	+4
O+	17	15	–2	26	19	–7	13	15	+2	11	8	–3
O=	13	12	–1	5	10	+5	11	10	–1	9	11	+2
O–	16	12	–4	1	3	+2	9	10	+1	17	13	–4
P	10	18	+8	1	3	+2	3	5	+2	47	48	+1
Fatness[b]												
1	0	1	1	-	-	0	2	4	+2	18	20	+2
2	8	11	+3	4	6	+2	37	48	+11	17	15	–2
3	53	47	–6	37	38	+1	53	45	–8	27	30	+3
4	36	39	+3	56	51	–5	7	3	–4	32	26	–6
5	1	2	+1	2	4	+2	-	-	0	7	6	–1

[a] Conformation assigned by VIA was measured on the15 point scale but only 7 points were used in accordance with UK classification.
[b] Fatness was also measured on the 15-point scale but converted to 5-points used in the UK.
[c] The Diff columns are the percentage point difference between the VIA and the 5 person panel.

2.4.9 VIA prediction of the proportion of high-value cuts

Saleable meat from a carcase can be divided (graded) into various value categories. For example, Pabiou *et al.* (2011b) divided meat into categories of very high-value (VHVC, which include the rib-roast, strip loin and fillet cuts), high-value, medium value, and lower value cuts. A larger proportion of total SMY% in the VHVC category is desirable, but in order for this to be evaluated, an accurate measure of both carcase composition and primal composition is required (Drennan *et al.* 2008). As shown in Table 2.3 and Table 2.4, EUROP classification accounts for much less variation in the LMY% of the VHVC than the total LMY%. Although VHVC are a small proportion (~7%), of total carcase SMY%, accurate prediction is essential for accurate carcase evaluation.

Breeding strategies aimed at maximizing the proportion of lean meat in high-value cuts need to capitalize on underlying variation of the trait(s) segregating in the population. With the exception of muscular hypertrophy found in some breeds of cattle (Arthur 1995), variation in lean meat distribution throughout the carcase is low. One experiment reported that the CV in percentage of total lean meat occurring in higher priced joints ranged from 4.8% in the Topside to 9.4% in the wing-rib in 643 steers of 10 British and continental breeds (Kempster *et al.* 1976). The level of variation in lean meat distribution was similarly low (CV < 10%) in a more recent dissection trial conducted in 2006, and involving 22 steers of UK Aberdeen Angus and Limousin crossbreds (R.

Roehe, SAC unpublished results). This low variation presents a further challenge for VIA because very precise predictions would be required to detect such small differences between carcases. But again, the difference between SMY% and LMY% is important, because the variation in SMY% throughout the carcase will be a combination of the variation in LMY% and FAT% for any given cut.

Besides predicting SMY%, FAT%, BONE% and classifying carcases according to the EUROP grid, VIA can also measure the distribution of meat within a beef carcase (personal communication, Axel Hinz E+V GmbH, Germany), which cannot be achieved by a human classifier; this differentiates VIA from visual appraisal. If VIA can accurately predict the proportion of meat in cuts of different value, there is potential for a pricing mechanism to reflect both the total SMY% and the SMY% of individual carcase cuts (assuming that the differential value of cuts such as the sirloin, cube-roll and fillet is related to consumer demand and willingness to pay).

Published results detailing the ability of VIA to directly predict the SMY% and composition of primal joints are scarce. One experiment, involving 232 steers (Pabiou *et al.* 2011b) included VIA variables + cold carcase weight (CCW) in a prediction model that accounted for 71% (RSD = 1.90%), 72% (RSD = 1.70%), and 75% (RSD = 0.90%) of the variation in carcase SMY%, FAT% and BONE% respectively. The corresponding accuracies using VIA-predicted EUROP + CCW in the model were 74% (RSD = 1.79%), 63% (RSD = 1.93%), and 63% (RSD = 1.09%), for SMY%, FAT% and BONE% respectively. Pabiou *et al.* (2011b) also reported accuracies for yield of VHVC as a percentage of carcase weight of R^2 = 45% (RSD = 0.52%) using VIA-assessed EUROP + CCW. When excluding EUROP but retaining other VIA variables along with CCW they reported an R^2 = 52% (RSD = 0.48%) for yield of VHVC. The similarity between R^2 and RSD values associated with prediction of SMY% based on VIA variables directly or VIA-assessed EUROP classification indicates that both prediction systems can be used, which is important because the EUROP classification is required by law. The reduction in R^2 associated with prediction of VHVC yield in comparison to carcase SMY% highlights the difficulties of predicting yield of VHVC yield as also shown in previous experiments (Table 2.4) and is again similar when using VIA variables directly or by using the VIA assessed EUROP classes in the prediction model.

2.4.10 Shortcomings of VIA

Whilst VIA might appear to offer solutions for many of the problems encountered in carcase evaluation and benchmarking, it is not perfect. Most researchers have found that VIA-predicted conformation has a higher correlation to the reference panel values than VIA-predicted fat class (Madsen and Thodberg 1994; Madsen *et al.* 1996; Augustini *et al.* 1997; Branscheid *et al.* 1999) while others have found the correlations to be similar for both conformation and fat class prediction (Allen and Finnerty 2000; Sonnichsen *et al.* 2006). It is important to remember that EUROP conformation and fatness are not always accurate determinants of carcase composition. Results of Pabiou *et al.* (2011b) show that performance of VIA for carcase SMY% prediction was not as accurate for heifers (R^2 = 36%, RSD = 3.26%) as for steers (R^2 = 75%, RSD = 1.77%). Those authors also reported that in Belgian Blue x Dairy steers VIA estimates were subject to significant negative bias for total fat weight (–3 kg, $p < 0.05$) and that VIA underestimated the weight of lower value cuts in crossbred steers of dairy breeds (–5.4 kg, $P < 0.001$) (Pabiou *et al.* 2011b). It is important to note that the VBS 2000 currently does not estimate carcase FAT% by default and the VIA images were subject to further processing by E+V GmbH to obtain FAT% (personal communication, T. Pabiou, Irish Cattle Breeders Federation).

2.4.11 VIA and the distribution of fat

All whole-side VIA machines (except the MACS) collect information from the lateral view of the carcase only, so predictions of fat class or FAT% are based on subcutaneous fat coverage. Although FAT% is not currently an output of VIA machines, it is needed if the SMY% is to be determined. Carcase FAT% is the most variable carcase component and is also most difficult to measure by VIA because fat is deposited in several different locations in the carcase some of which are not superficial. These sites include subcutaneous fat (SF), intermuscular fat (IF), intramuscular fat (IMF) and kidney, knob and channel fat (KKCF) depots (Kempster *et al.* 1982a). A further complicating challenge is that the partitioning of total carcase fat between these depots varies by breed, sex, maturity, and diet (McPhee *et al.* 2009). As an example of the breed effect, the SF:IF ratio is considerably higher in beef steers than dairy crossbred steers so prediction of carcase composition in dairy cattle will be less stable if based on

the SF depot only (Fisher and Bayntun 1984). Fortunately, SF is usually the largest depot and the easiest to trim off (Kempster 1981), but being subcutaneous, it is also susceptible to uncontrolled/spurious removal during hide pulling. In the UK and some other countries, KKCF is removed prior to carcase evaluation, IMF (marbling) can add value in certain markets, and IF is often removed during carcase break-down.

2.4.12 Other constraining factors

Two further constraints for the implementation of VIA are, first, the space requirements for installation, and, secondly, the fact that small abattoirs will face a larger cost relative to throughput than their larger counterparts so that their uptake of a VIA system may be lower. VBS 2000, for example, requires 9.2 M^2 of floor space. As many abattoirs were not designed to have extra space in the slaughter line, making the space for a VIA system can be expensive. A more compact VIA system may be a solution to the first constraint, the VIAscan system is more compact, but it has not gained wide acceptance in Europe. The solution to the second constraint relates to economies of scale either achieved by increasing throughput or reducing the processing cost per carcase, and to the availability of appropriate business, purchase, or other support models that may facilitate the acquisition of VIA technology.

2.4.13 Further development of VIA

Current commercial VIA systems were developed mainly in the 1990's, but it is now possible to capture images in far greater resolution at a much lower cost and to handle much larger volumes of data. Oliver *et al.* (2010), for example, used two-dimensional still digital photographs and image analysis software to predict the weight of meat in four categories for 91 young bulls in Spain, and showed that image analysis accounted for more variation in cut weight than hot carcase weight alone, or combined with visual conformation scores that were assigned by two classifiers (Table 2.11). The young bull carcases in the experiment of Oliver *et al.* (2010) were very lean (FAT% of 9.2%) and the prediction equations were for saleable meat weight rather than the more informative SMY%. The comparison between image analysis and [S]EUROP conformation without fat class may be problematic if applied to other types of cattle where the FAT% is much higher. Unfortunately Oliver *et al.* (2010) did not apply their image analysis techniques

to predict FAT%. In a pilot trial involving 29 cross-bred steer and heifer carcases under abattoir conditions, the area of fat on the carcase explained between 1 and 2% of the variation in total fat weight in the half carcase (Prieto *et al.* 2009c). With addition of linear dimensions from the images, models could account for 48% of the variation in fat weight from the half carcase (Prieto *et al.* 2009c).

Table 2.11 Prediction of different cut weights from hot carcase weight (HCW) and its combination with visual conformation ([S]EUROP) or image analysis parameters on ventral and lateral digital photographs of 91 young bull half carcases with a mean carcase weight of 174.3 kg (range 101.4-225.3 kg). Derived from Oliver *et al.* (2010).

	Hot carcase weight (HCW)		HCW + Visual conformation		HCW + Image analysis	
Cut class (kg ± SD)	R^2 (%)	RSD	R^2 (%)	RSD	R^2 (%)	RSD
Extra [a] (3.4 ± 0.57)	50	0.4	66	0.33	79	0.26
First [b] (77.0 ± 11.74)	80	5.24	93	3.06	94	2.74
Second [c] (9.3 ± 1.41)	83	2.26	78	0.65	84	0.55
Third [d] (34.1 ± 5.52)	72	0.75	87	2.01	90	1.73
Total meat [e] (123.9 ± 18.74)	84	7.59	93	4.76	96	3.84

Note: The mean ± SD of each class are given in parentheses.
[a] Extra (included the fillet only).
[b] First (included strip-loin, rump, knuckle, rump tail, chuck, silverside, topside, eye-round, shoulder, chuck tender, heel).
[c] Second (included fore shin, shank, blade).
[d] Third (included neck, flank, brisket).
[e] Total meat is the sum of all the cut classes from a half carcase.

A common method of determining the three-dimensional carcase shape is measuring the extent to which bands of structured light are deformed by surface curvature (Vuylsteke 1990; Yang 1993). Using triangulation it is possible to calculate the profile of the carcase because the angles of the light projector and camera are fixed. Structured light is used by the BCC-2 and the VBS 2000 (Figure 2.1). Refining this method of obtaining three-dimensional imaging may enable VIA to be much more compact.

Photogrammetric stereo is an alternative method that involves mounting two cameras at a known distance apart so that two photographs of the same scene are produced and a three-dimensional image developed by overlaying the two images. This approach has been used to create three-dimensional images of live pigs (Wu *et al.* 2004). An advantage of photogrammetric stereo is that it can be performed in normal light or in combination with a structured light pattern (Mazaheri and Momeni 2008).

As new technologies such as stereoscopic infrared cameras (Menesatti *et al.* 2007) and three-dimensional high-definition video cameras become smaller and more affordable, they may be suitable for online application within VIA systems. EU regulations might require new validation trials if the technology used by systems such as the VBS 2000,

BCC-2 and Normaclass MAC were to change. This could act as a disincentive to upgrading the technology, yet Carometic A/S is endeavouring to upgrade the camera and server systems in the BCC-2 so that a second set of prediction equations can be customized for individual plant management requirements (personal communication, Henrik Andersen Carometic A/S, Denmark).

2.5 Summary and conclusions for VIA

The main conclusions of this review with respect to VIA for carcase evaluation are as follows:

1. VIA can be applied either to the whole carcase before entry to the chiller or to the rib surface of the quartered carcase after chilling.

2. The rib surface approach is used extensively in the USA and Canada.

3. For markets such as the EU, where there is a need to automate visual assessment of whole carcases the whole-side approach to VIA is preferred.

4. VIA is objective, fast, and in the case of whole-side systems, is totally automated.

5. Whole-side VIA is more versatile because it is performed before carcase cutting and is not reliant on a specific quartering position.

6. VIA systems tend to be able to account for more variation in SMY% with greater repeatability than visual assessment methods.

7. The use of the expert panel for validating VIA for carcase classification on the EUROP grid is not representative of everyday operating conditions where a single classifier is used.

8. The use of CT scanning to determine carcase composition (e.g. LMY%, FAT%, muscle distribution, and muscle-to-bone ratio) would be a more robust and informative method of validating VIA than a panel of five trained assessors.

9. Regression equations can be used to determine the ability of VIA to predict SMY%, but the accuracy and precision of predictions are heavily dependent on the SMY% variation in the sample carcases, the level of fat trimming, and the variability in fat trimming.

10. An overall appraisal of the accuracy of whole-side VIA has been lacking to date, the summary provided in this review shows that the overall predictive ability of VIA is remarkably good with median R^2 values of 70%, 80%, 82%, 90% and

83% for SMY%, FAT%, BONE% EUROP conformation, and fatness, respectively.

11. The ability to measure the distribution of SMY% within high value portions opens up the possibility of rewarding producers on that basis. Producer remuneration based on LMY% or SMY% can be used to help producers improve carcase quality and improve industry efficiency. Future research efforts should focus on improving the accuracy and precision with which VIA predicts FAT% and LMY% for purposes of carcase evaluation.

12. Very few recent investigations have tested VIA variables such as lengths, widths, areas and ratios as predictors of LMY%. As a result, it is not clear whether predictions of carcase LMY% or indeed the yield of high value cuts could be improved through the use of other VIA variables in prediction equations.

2.6 Meat eating quality

At the point of evaluation, carcases are still treated as a commodity product as opposed to saleable meat. The key difference is that the qualitative value aspect of a carcase can be very different to that of the saleable meat, particularly in carcase evaluation systems that do not evaluate meat quality. For example, the EUROP carcase classification system does not measure meat eating quality or even SMY%, rather conformation and fatness are the parameters. The correlation between EUROP classification and SMY% is variable for a number of reasons and the relationship between EUROP and consumers' perception of meat quality is not known. At the point of purchase the eating quality of meat is unknown. Further preparation is required before consumption and the ultimate assessment of eating quality by the consumer. As a result, consumers must predict eating quality at the point of purchase based on any available information at hand. The total food quality model (Grunert 2005) explains consumer satisfaction by comparing expectations formed at the point of purchase with the actual eating experience. If the actual eating quality (determined during consumption) matches or exceeds expectations formed at purchase, the customer is satisfied and is more likely to repeat their purchase. Considering a whole-meat product, there is basic expectation of "wholesomeness" which relates to nutritional quality, and microbiological and chemical safety (Gellynck *et al.* 2006). The size, shape and packaging of meat also affect consumers purchase decisions due to a convenience element (Grunert 2005).

There are many interactions between meat production, processing and preparation parameters that influence components of meat quality which are beyond the scope of this review, but are often topics of investigation in the literature. The following sections will focus on intrinsic components of whole meat such as the visual aspects (e.g. colour of lean meat) and the palatability aspects (such as tenderness, juiciness and flavour) rather than the consumer perceptions of meat quality *per se*, the role of science in consumer perception has been reviewed by Troy and Kerry (2010). Visual factors such as lean meat and fat colour as well as the amount of intramuscular, intermuscular or subcutaneous fat present are also used by consumers to infer eating quality at the point of purchase (Grunert *et al.* 2004).

2.6.1 Meat colour

The redness of meat is used by consumers to infer freshness (Mancini and Hunt 2005) but the correlation between colour traits and meat eating quality is low (Moore and Young 1991). Some beef carcase evaluation systems such as Meat Standards Australia (MSA) recognise the importance of meat colour to consumers and evaluate meat colour in the chiller (Polkinghorne and Thompson 2010), but it is not clear if meat colour is a useful predictor of MQ4 which is the scale of meat eating quality used in the MSA system (Watson *et al.* 2008). The most common way of measuring colour instrumentally is with a hand-held chroma meter such as a Minolta or Hunter-LAB instrument but meat colour can also be measured from digital images (Larraín *et al.* 2008). Chroma meters measure colour as coordinates in a three-dimensional colour sphere with a lightness (L*) axis ranging from black (0) to white (100), a red-green (a*) axis where positive values are more red and negative values are more green, and a blue-yellow (b*) axis where positive values are more yellow and negative values are more blue (Hunt *et al.* 1991). The three colour coordinates taken on a sample using a chroma meter (such as a Minolta CR-410) are used to describe the sample's colour in a three-dimensional colour space. In meat science, the L*, a* and b* values are often used separately, but the hue angle, and chroma (or saturation), are also calculated from the L*, a* and b* values using standard formulas (for example see Ripoll *et al.* (2011)). Hue angle is the proportion of redness and yellowness indicated by the angle at which a vector radiates into the red-yellow quadrant (Liu *et al.* 1996). Chroma relates to the vividness of colour, where low values represent a lack of colour (Ripoll *et al.* 2011). Some researchers have proposed consumer acceptability thresholds for fresh lamb meat colour (Hopkins 1996; Khliji *et al.* 2010). Khliji *et al.* (2010) proposed that a minimum L* of 44 and an a* of 14.5 is required for 95% of consumers to find lamb meat acceptable in terms of colour; but there are few such thresholds in the published literature.

Meat colour can be affected by many aspects of production, processing and packaging. One of the more common factors afflicting meat colour is the condition of dark cutting. Meat is described as dark cutting when the colour is noticeably darker than normal due to high ultimate pH. Dark cutting is usually as a result of ante-mortem stress depleting cellular glycogen reserves (Kreikemeier *et al.* 1998). Dark cutting meat has a shorter

shelf life because bacteria can survive at the elevated pH and is also less visually acceptable to consumers than normal coloured meat; although differences in terms of tenderness, juiciness and flavour can appear minimal (Viljoen *et al.* 2002; Bass *et al.* 2008). Meat surface colour is also affected by the oxidative state of myoglobin, where de-oxymyoglobin (purple colour) quickly becomes cherry red oxymyoglobin on exposure to oxygen which oxidizes to the brown metmyoglobin (Mancini and Hunt 2005). Packaging also has a large effect on meat colour. The review by McMillin (2008) details the development and use of modified atmosphere packaging of meat to improve colour stability. There is also a relationship between colour and IMF, because meat with a higher IMF content appears lighter (Fiems *et al.* 2000). The level of IMF is valued in some markets such as Japan and Korea (Thompson 2004; Polkinghorne and Thompson 2010).

2.6.2 Fat

Besides IMF, the level of subcutaneous or intermuscular fat can also affect consumer perceptions, particularly in lamb (Jeremiah *et al.* 1993). As well as having an impact on pre-purchase meat appearance, IMF also plays a role in the palatability of meat, where increasing levels are generally associated with improved eating quality (Hocquette *et al.* 2010). Although the interaction between IMF and palatability is not well understood, one theory is that a higher percentage of IMF results in a lower percentage of muscle fibres and connective tissue resulting in a lower density meat that has less resistance to chewing (Nishimura *et al.* 1999; Warner *et al.* 2010). The effect of IMF on juiciness is thought to be due to the stimulation of salivation leading to a lubrication effect during mastication (Thompson 2004). Levels of IMF can range from very low < 1% in venison (Purchas *et al.* 2010) and < 2% in some breeds of sheep (Lambe *et al.* 2008; Navajas *et al.* 2008; Lambe *et al.* 2011) to very high in some breeds of cattle (Hocquette *et al.* 2010). *M. longissimus lumborum* from lamb chops purchased at retail outlets in the UK had an average of 3.20% (Angood *et al.* 2008). One experiment in Scotland reported IMF in beef to range between 1.65-5.70% with an average of 3.89% (Prieto *et al.* 2011). Several authors have recommended minimum IMF% for acceptable eating quality, which is about 3-4% for beef (Savell and Cross 1986) and greater than 5% for lamb (Hopkins *et al.* 2006), although approximately 2% is thought to be sufficient in UK markets (personal communication, J.D. Wood, University of Bristol). As opposed to the

role of IMF in the palatability aspects of meat quality, the review by Wood *et al.* (2008) discusses the role of IMF composition in terms of nutritional meat quality. Despite variation within and between species, IMF could be considered a key element of meat quality as it is affects the nutritional quality, visual appeal and palatability of meat.

2.6.3 Tenderness

Extensive consumer testing in several countries has identified tenderness as the major factor contributing to a positive meat eating quality experience (Huffman *et al.* 1996; Bickerstaffe *et al.* 2001; Miller *et al.* 2001; Maltin *et al.* 2003; Price *et al.* 2008). Tenderness is defined in three stages, the ease of teeth penetration on the first bite, the ease at which the meat becomes fragmented and thirdly, the amount of residual material left after chewing (Weir 1960). The tenderness of cooked meat can be assessed either with humans via a trained or untrained sensory panel, or using mechanical laboratory-based shear force tests. Typically shear force tests such as the Warner-Bratzler (Bratzler 1949), Volodkevich (Volodkevich 1938), MIRINZ tenderometer (Macfarlane and Marer 1966) or rapid slice shear force (Shackelford *et al.* 1999a; Shackelford *et al.* 2004) are applied across the muscle fibre axis on samples of a pre-defined dimension. Higher peak shear force values are indicative of tougher meat in all tests. Although shear force devices only approximate actual tenderness, they are used in place of sensory panels to minimize cost. Ideally a high correlation between shear force tests and tenderness as defined by a sensory panel is desirable. An important factor affecting tenderness is the degree of "doneness" of the meat resulting from varying end point cooking temperatures (Luchak *et al.* 1998; Purchas *et al.* 2010). When measuring tenderness, samples must be cooked to a constant temperature to eliminate the "doneness" effect before tenderness or shear force can be determined. Table 2.12 lists correlations obtained between shear force instruments and tenderness as determined by a trained sensory panel. As expected the peak shear force values produced by all instruments are negatively correlated to trained sensory tenderness, but there is considerable variability between experiments. Correlations between shear force measures and a trained sensory tenderness score may be improved by using the entire force deformation curve (force over time) in a multivariate analysis approach (Hildrum and Narum 2006). Correlations between shear force instruments and consumer panels are much less common, although Wheeler *et al.* (2004) reported a correlation of –0.92 between 14d shear force and an a mean

tenderness score from 16 un-trained consumer panellists. The relationship between trained sensory panellists and untrained consumers is not necessarily strong (Lorenzen *et al.* 2003; Aalhus *et al.* 2004), possibly due to differences in training, and because tenderness varies within and between muscles. The correlations between muscles are not necessarily strong, leading some researchers to conclude that tenderness is muscle specific (Aalhus *et al.* 2004; Polkinghorne and Thompson 2010). There are several biological factors affecting meat tenderness, for instance, chilled aging of meat increases tenderness due to post mortem proteolysis by the calpain system which breaks down meat structure (Koohmaraie *et al.* 1991). The calpain system is a family of Ca^{2+} dependant cysteine proteases that are largely responsible for the tenderization of meat during the aging process (Koohmaraie 1996). Of the calpains, Geesink *et al.* (2006) reported that μ-calpain is largely responsible for the post mortem proteolysis of muscle proteins. The rate of post mortem proteolysis also varies by species, venison for example, has a fast tenderization rate compared to beef (Barnier *et al.* 1999; Farouk *et al.* 2007). Other factors such as ultimate pH and temperature also play an important role in tenderization of meat. A rapid drop in muscle temperature before sufficient pH decline *post mortem* can result in an early onset of rigor and "cold shortening" which is linked to toughness in meat (Dransfield and Rhodes 1976).

Table 2.12 A selection of correlation coefficients (*r*) between shear force and tenderness (as assessed by a trained sensory panel) for a range meat types and shear force tests taken from the published literature.

Reference	Samples	Shear force test[a]	Aging time (days)[b]	Correlation
Safari *et al.* (2001)	60 lambs	WBSF	7	–0.71
Muchenje *et al.* (2008)	34 steers	WBSF	2	–0.23–-0.48
Ross *et al.* (2009)	150 steers and heifers	SSF	3	–0.60
	150 steers and heifers	SSF	14	–0.47
	150 steers and heifers	Volodkevich	14	–0.47
	131 steers and heifers	MIRINZ	14	–0.58
Lambe *et al.* (2011)	40 Texel lambs	Volodkevich	7	–0.36
Chambaz *et al.* (2003)	64 steers	WBSF	14	–0.43
Peachy *et al.* (2002)	117 bulls and steers	WBSF	7	–0.73
		MIRINZ	7	–0.71
Shackelford *et al.* (1995)	16 steers	WBSF	14	–0.73
Shackelford *et al.* (1999a)	479 steers and heifers	WBSF	14	–0.77
	479 steers and heifers	SSF	14	–0.82
Sullivan and Calkins (2011)	Meta analysis	WBSF	Various	–0.84

[a] WBSF = Warner-Bratzler shear force, SSF = rapid slice shear force, MIRINZ = MIRINZ tenderometer.
[b] The duration of chilled aging before testing.

2.6.4 Juiciness

According to Weir (1960), the definition of juiciness consists of two stages, firstly the wetness experienced during the first few chews and secondly the sustained sensation of

moistness resulting from the stimulatory effect of fat on the salivary glands. Despite this, juiciness is assessed as a single attribute (Dransfield *et al.* 1984). Excessive moisture loss results in dry meat, the ability of meat to retain moisture (or water holding capacity, WHC) is therefore a trait of interest, although the relationship between juiciness and WHC is inconsistent (Winger and Hagyard 1995). The amount of moisture lost during cooking is a common proxy indicator of WHC as is purge (sometimes called drip loss) which is defined as the amount of moisture lost in packaging (Wiklund *et al.* 2001). The filter paper press method (Hamm 1986) is another method of determining WHC where expressed juice is calculated by dividing the juice-stained area on filter paper by the sample weight after pressing for a set time between two flat plates. The correlation between cooking loss and juiciness is one of the most common measures of WHC reported in published literature (Table 2.13).

Table 2.13 Published correlation coefficients (*r*) between cooking loss and juiciness as assessed by a trained sensory panel.

Reference	Samples	Muscle	Cooking method[a], Temperature	Correlation
Stevenson *et al.* (1992)	20 Red deer stags	*Longissimus thoracis*	Dry, 65°C	–0.47
		Semimembranosus	Dry, 65°C	–0.58
Safari *et al.* (2001)	60 lambs	*Longissimus thoracis*	Dry, 76°C	–0.32
Vipond *et al.* (1995)	24 lambs	*Longissimus thoracis*	Dry, 65°C	–0.40
		"Leg steak"	Dry, 70°C	–0.40
Schönfeldt and Strydom (2011)	61 beef carcases	*Triceps brachii*	Moist, 70°C	–0.57
		Biventer cervicis	Moist, 70°C	–0.51
		Vastus lateralis	Moist, 70°C	–0.46
		Longissimus thoracis	Dry, 70°C	–0.45
		Psoas major	Dry, 70°C	–0.33

[a] "Dry" refers to open grilling and roasting, "Moist" refers to roasting in a closed dish.

2.6.5 Flavour

Flavour of cooked meat is highly subjective; there are few methods of measuring flavour besides a sensory panel. One alternative is the "Electronic Nose" based on gas chromatography as described by Ghasemi-Varnamkhasti *et al.* (2009). The "electronic nose" technology is still relatively new and there are few reports containing correlations to sensory panels. One report has detailed breed differences using this technology and reported that Belgian Blue bull beef generated more odour-active compounds than Limousin or Aberdeen Angus bull beef (Machiels *et al.* 2004). Generally speaking, panellists describe the taste (such as "sweet" or "bitter") and flavour of meat such as "milky", "fishy, "flavour" and "abnormal flavour" on a hedonic scale (Sañudo *et al.* 2007; Maughan *et al.* 2012). Many factors are known to affect flavour in meat; Calkins

and Hodgen (2007) review some of the key compounds contributing to meat flavour. The importance of meat flavour to the overall eating quality experience is difficult to define and is species specific, the often-intense flavour of game meats (Hoffman *et al.* 2009) is an example of this, and flavour may be more important than tenderness for consumer preference in lamb (Sañudo *et al.* 2007). Schreurs *et al.* (2008b) review how forage-based diets affect sheep meat flavour. The effects of some poly-unsaturated fatty acids on meat flavour are discussed by (Sañudo *et al.* 1998; Wood *et al.* 2008). Thompson (2004) reported that the correlation between IMF% and beef flavour is 0.41 after adjusting to a constant tenderness, although in beef, flavour does not differ between muscles in the same way as tenderness. It has been reported that, there is between three and four times more variation in beef tenderness than beef flavour (Shackelford *et al.* 1995) and it is important to note that meat flavour can be easily influenced through the use of marinades and bastings (Van Wezemael *et al.* 2012).

2.6.6 Consumer willingness to pay for meat eating quality

The concept of paying more for better eating quality is not new, in terms of beef, table cuts such as the fillet, sirloin, rib-eye and rump command premium prices per kilo over lower quality cuts which are often minced or diced (Pabiou *et al.* 2011a). At an animal level there is a possibility that carcase evaluation could be based on SMY% and factor in higher yields of high value cuts if measurement techniques are practicable. Considering consumers' willingness to pay (WTP) at an industry level, there is a need to improve meat product consistency to minimize the chance of a poor eating quality experience and to capitalize on price premiums associated with higher prices and higher repeat purchases (Grunert 2005). This relies on the assumption that consumers are willing to pay for superior eating quality. There is a growing body of evidence that has investigated consumers' WTP for superior beef eating quality. Perhaps the largest experiment investigating the magnitude of the premiums is the research of Lyford *et al.* (2010) who investigated WTP based on the MSA system. These authors collated WTP from 6718 untrained Australian, American (USA), Japanese and Irish consumers who had a preference for a medium level of "doneness". Interestingly consumers in all countries would pay twice as much for "good everyday" (3-star) beef than "unsatisfactory" (2-star) beef (Table 2.14). For example, using the WTP results for Australian consumers in Table 2.14 reported by Lyford *et al.* (2010) if a good everyday

steak was priced at £15.99 per kg then if it was of unsatisfactory quality it would be worth £9.11 per kg, conversely if it was better than every day or premium it would be worth £24.14 per kg or £33.60 per kg respectively. The relationship between WTP and meat eating quality was linear in Australia, Japan and the USA with Irish consumers showing the smallest increase in WTP for quality. In Japan the WTP for 4- and 5-star beef was curvilinear with consumers willing to pay increasingly higher premiums for increasing beef eating quality. In another experiment where South African and Australian consumers were compared, the WTP was again similar across countries (Table 2.14) (Thompson *et al.* 2010). One other interesting finding in both the work of Lyford *et al.* (2010) and Thompson *et al.* (2010) as well as other reports was the consistent interaction between age and WTP for beef eating quality in all countries investigated, where younger consumers were more willing to pay a higher premium than older consumers (Lusk *et al.* 2001; Lyford *et al.* 2010; Thompson *et al.* 2010).

Table 2.14 Mean and standard deviation of the price that consumers are willing to pay for beef eating quality relative to 1.00 for 3-star "good every day" quality from consumer sensory evaluations in different countries.

		Quality grade			
Country	Number of Consumers	2-star unsatisfactory	3-star good everyday	4-star better than everyday	5-star premium
Reference: Lyford *et al.* (2010)					
Australia	2116	0.57 (0.23)	1.00	1.51 (0.32)	2.10 (0.61)
USA	1338	0.56 (0.20)	1.00	1.64 (0.44)	2.37 (0.80)
Japan	1471	0.48 (0.16)	1.00	1.69 (0.38)	2.86 (1.00)
Ireland	960	0.49 (0.21)	1.00	1.46 (0.31)	1.97 (0.55)
Reference: Thompson *et al.* (2010)					
Republic of South Africa	545	0.47 (0.24)	1.00	1.55 (0.48)	2.35 (1.09)
Australia	533	0.44 (0.20)	1.00	1.51 (0.29)	2.15 (0.62)

Note: Standard deviations are included in parentheses.

There is little information available on WTP for lamb, except for a pilot trial conducted in Scotland (n = 85 consumers for lamb and n = 100 consumers for beef) (Craigie 2011) 90% of consumers were prepared to pay more for guaranteed eating quality and the premium was approximately 10% for both lamb and beef. Around 90% of those consumers also supported the concept that farmers and meat processors should be rewarded for producing lamb or beef with a superior eating quality. The problem with this analysis was that there was no indication of what a guarantee might mean, although the questions posed to the consumers were expressed in relation to a six products sourced from retail outlets which the consumers had just assessed in a hall test. A further criticism of many WTP analyses is that consumers do not actually purchase the meat in the protocol. Results reported by Polkinghorne *et al.* (2008) show clearly that

consumers are willing to pay for a quality guarantee when they actually purchase the meat. Although the MSA system has been demonstrated to add value, it is possible for retailers to purchase beef under the MSA scheme and re-brand it as their own premium brand. This undermines the MSA system by removing the association between MSA ratings and the intrinsic attributes of the product. The only brand of beef to carry a tenderness guarantee in the USA is the "Rancher's Reserve" brand from Safeway which is underpinned by a rapid slice shear force test developed by Shackelford *et al.* (1999) (personal communication, M. Koohmaraie, IEH Laboratories Inc.). It is not clear how the tenderness guarantee translates into carcase evaluation or producer premiums. WTP based on eating quality is a useful concept; but eating quality indicators based on reliable, published methods of predicting meat eating quality remain illusive.

There are several drawbacks associated with using sensory panels or mechanical tenderness tests to judge meat eating quality. Firstly, each test requires destruction of the product; secondly, trained sensory evaluations are expensive and are not necessarily representative of consumer perceptions of meat eating quality (Lorenzen *et al.* 2003). Thirdly, sensory parameters such as tenderness, juiciness and overall liking tend to be highly correlated when assessed by untrained consumer panels (Hwang *et al.* 2008; Thompson *et al.* 2010). Furthermore, none of these methods are suitable for use at line speed under abattoir conditions and are therefore unsuitable for carcase evaluation purposes, with perhaps the exception of rapid slice shear force which was intended to operate at line speed for beef (Shackelford *et al.* 1999a). Consequently, there has been much interest from a number of countries in the non-destructive prediction of meat eating quality using the MSA system (Polkinghorne and Thompson 2010), but to date the MSA system has not gained wide acceptance outside of Australia. Methods for predicting meat eating quality parameters need to be safe, non-destructive accurate, precise and informative to be suitable for carcase evaluation purposes.

2.7 Visible-near infrared spectroscopy

Visible-near infrared (NIR) spectroscopy applied to whole meat has been identified as technology that is possibly suitable for predicting meat eating quality. NIR has been widely investigated with respect to its ability to predict instrumentally derived meat quality traits (such as shear force, pH and colour), sensory properties and chemical

composition as described by three excellent reviews (Prevolnik *et al.* 2004; Prieto *et al.* 2009a; Weeranantanaphan *et al.* 2011). NIR spectroscopy is a spectroscopic method that utilizes the visible (~400-700 nm) and near infrared (~800-2500 nm) regions of the electromagnetic spectra for determining chemical composition of a given sample. NIR has two main modes of application; transmittance mode where light is shone through a sample (usually in a cuvette) into a detector, and reflectance mode where light is shone at a sample and a detector measures the reflected light (Osborne *et al.* 1993). Transmittance NIR is more suited to non-particulate liquids whereas, reflectance spectroscopy is more suited to opaque solids including whole meat (Osborne and Fearn 1986). Furthermore, there are two types of reflectance termed "specular", where radiation is reflected off the surface (like a mirror) and "diffuse" where light that enters the sample becomes diffused by random reflections, refractions and scatter at further interfaces within the sample (Osborne and Fearn 1986). The same authors explain that diffuse reflectance spectra of biological samples relate to discrete structural components or particles, as well as to molecular structures.

2.7.1 Mechanics of NIR spectroscopy

Absorption of electromagnetic radiation at specific wavelengths relates to changes in a molecule's vibration state (Cardarelli 2008). Upon exposure to electromagnetic radiation, particular molecular moieties absorb radiation and are excited to a higher energy level, (known as a transition). Depending on the transition state (how excited the molecule is), first, second and third overtones are detected in the resulting reflected spectra (Weyer 1985). For example, the first, second and third overtones for H_2O at room temperature are detected at ~1458, ~980 and ~744 nm respectively, the O-H bonds vibrate in a different way upon exposure to the discrete electromagnetic wavelengths (or ranges of wavelengths) (Osborne *et al.* 1993). In a NIR spectrometer, reflected electromagnetic radiation is detected by a photoelectric diode or similar device (Workman 2004). Particular molecules and moieties absorb electromagnetic radiation; the amount of energy reflected is lower at specific wavelengths where electromagnetic radiation has been absorbed by particular molecules in the sample. Because different moieties have different bonding structures, the specific wavelength that is absorbed varies accordingly, hence NIR electromagnetic radiation can be used to partially determine the chemical composition of a sample. In order for an NIR spectrometer to

determine the specific wavelengths where absorption has occurred, a reference measure is needed. A white calibration tile that reflects 100% of the radiation at all NIR wavelengths is used as a reference measure, after scanning, sample reflectance is expressed as a percentage of the reference (Workman 2004). The absorbance is the difference between the sample reflectance and the calibration reflectance where absorbance = log(1/Reflectance) (Naes *et al.* 2002). The relationship between reflectance and absorbance is complicated by scatter effects and path length differences resulting from interactions between light and structural properties of the sample (such as particles or droplets) (Osborne *et al.* 1993). Scatter effects add noise to NIR spectra and complicate analysis because not all missing energy is absorbed. In order to improve the signal to noise ratio, various mathematical pre-treatments can be applied to the spectra that aim to separate the chemical absorbance from the physical absorbance that is mainly responsible for the noise.

2.7.2 Scatter effects and data pre-treatments

There are two types of scatter effects, termed additive and multiplicative (Esbensen *et al.* 2009). Additive effects are seen as different y-axis offsets for different spectra while multiplicative scatter effects are seen as peak intensity dependant spread between different spectra. Plotting individual spectra against the average spectra enables the analyst to determine whether additive or multiplicative scatter effects are present and to decide on an appropriate pre-treatment (Geladi *et al.* 1985). The mathematical pre-treatment required depends on the type of scatter effects encountered. Many mathematical pre-treatments can be deployed to remove samples or variables for example, variable (spectra-based) treatments include normalizing, weighting, smoothing (using derivatives), baseline correction and multiplicative scatter correction (MSC) which can correct additive, multiplicative or both types of scatter effects (Esbensen *et al.* 2009). Sample-based pre-treatments can include mean centring and variable weighting (Beebe *et al.* 1998; Duckworth 2004). A full explanation of all pre-processing techniques used to prepare data for analysis can be found in (Beebe *et al.* 1998) and the typical NIR pre-treatments used in meat applications for a number of recent experiments are provided by Weeranantanaphan (2011). It is worth noting that the predictive ability of NIR in meat is seldom attributed to the spectral pre-treatment

alone, as other factors such as the precision of the reference method have a much larger effect on the development of a calibration model.

2.7.3 Advantages of NIR spectroscopy

Because reflectance NIR is conducted on the sample surface, minimal sample preparation is required before scanning (Murray and Cowe 2004). NIR spectra collection is rapid (1-2 seconds), a Quartz tungsten-halogen monofilament lamp can be used as a source of electromagnetic radiation (Workman 2004) and the collection of spectra does not destroy the sample. The fact that NIR spectroscopy can be used to measure several properties simultaneously provided the models have been developed is also advantageous. These properties render NIR spectroscopy suitable for testing the composition and properties of a range of biological materials, including meat. Conventional analyses of meat quality parameters such as tenderness, juiciness and flavour require destruction of the sample. For example, determination of instrumental meat tenderness requires removal of the meat from the carcase, joint, package etc., cooking in a standardized manner, preparation of the cooked sample into uniform blocks and shearing with a blade attached to a load cell (Purchas and Aungsupakorn 1993). Furthermore, in order to determine sensory properties of meat, a trained sensory panel is required and to determine the fatty acid profile of meat, gas chromatography is used (Sinclair *et al.* 1982). These methods are, slow, expensive and unsuitable for high throughput in an abattoir environment. Before NIR spectroscopy can replace these traditional methods, robust prediction equations need to be developed in the calibration phase of model development.

2.7.4 Calibrating NIR Spectroscopy to predict meat quality

Calibration requires collecting spectra (explanatory variables) on intact, homogenised or minced meat which are subsequently calibrated against the meat quality parameter of interest as the reference to determine the parameter (dependent variable) of interest. A model must be developed that is able to predict the reference value from the absorption profile of the spectra. Various statistical procedures are invoked to develop a prediction equation. Because there are often > 1000 explanatory (x) variables in NIR spectra and there is a high level of collinearity (x variables are intercorrelated), multivariate data

analysis techniques must be used. The most common method for developing a calibration model from NIR spectra is partial least squares regression (PLSR). PLSR solves the collinearity problem by first decomposing all x variables into orthogonal, linear principal components, which are subsequently used in a multiple linear regression approach (Naes *et al.* 2002). The optimal number of principal components is reached when the root mean square is minimized, but it is important to avoid over fitting, as this results in a data-dependant model with poor results (Naes *et al.* 2002). Other quantitative analytical approaches such as discriminant analysis and artificial neural networks are described by Kramer *et al.* (2004).

2.7.5 Validation of NIR prediction equations

It is necessary to validate models developed during the calibration phase to test their robustness in terms of accuracy and repeatability. Internal cross-validation and external validation (or prediction) are the two main methods of validating models (Esbensen *et al.* 2009). Cross-validation entails removing and predicting each record in a sequential manner. The advantage of cross validation is that every record is used both in the calibration and validation phases. In contrast, external validation involves the testing of models on naïve samples (samples that were not used in the calibration phase) (Naes *et al.* 2002). The disadvantage of this approach is that the data must be split into calibration (~70%) and validation (~30%) datasets. The validation set therefore cannot be used in the calibration phase which is disadvantageous in situations where the sample size is small. Depending on the intended use of the model, it is arguable that the cross-validation method is actually still part of the calibration phase rather than a form of model validation. This is because predictive performance on naïve samples ultimately determines the future performance of the models. Because of the increased interest in NIR for practical applications in the meat industry, external validation has become increasingly common in recent years (Weeranantanaphan *et al.* 2011).

2.7.6 Performance of NIR spectroscopy

There are many reports detailing the performance of NIR for predicting a variety of instrumentally-measured meat quality traits. The details of the experiments including the number and type of samples used, and the traits predicted using NIR spectroscopy

are listed in Table 2.15, Table 2.16, Table 2.17 and Table 2.18. Of the instrumental parameters predicted by NIR, Warner-Bratzler shear force is the most common, followed by the meat colour traits and cooking loss. Most reports are on Beef *M. longissimus thoracis* where the NIR spectra have been collected on intact samples, although some protocols have homogenized (Prieto *et al.* 2008) or minced the meat samples prior to collecting NIR spectra (De Marchi *et al.* 2007; Cecchinato *et al.* 2011). There are two main modes of shear force prediction, the first is a prediction of absolute values of tenderness on a continuous scale (Table 2.15, Table 2.16 and Table 2.17) although correlation coefficients above 0.6 are rare for predicting tenderness (Weeranantanaphan *et al.* 2011). The second type of prediction tests the ability (% of correctly classified samples) of NIR to classify meat into tenderness categories (Shackelford *et al.* 2005; Rust *et al.* 2008; Ripoll *et al.* 2008; Shackelford *et al.* 2012a; Shackelford *et al.* 2012b). The percentage of "correct" categorizations is usually much higher than predictions of a continuous variable because the variation within a category does not need to be discerned; therefore a less precise model is sufficient for categorization. The more tenderness categories are used, the lower the accuracy of correct classification. It appears that the tenderness classification approach is gaining more traction in USDA *select* grade carcases (Shackelford *et al.* 2012b), although thresholds used for the boundaries between categories tend to have a strong influence on the predictive ability of classification models, the lower the threshold, the higher the error rates (Rust *et al.* 2008; Shackelford *et al.* 2012a). In the US Meat Animal Research Centre research protocol for beef, there are two categories, "predicted tender" and "not predicted tender" where the median of the dataset is used as the threshold (Table 2.18). The mean shear force values of the "predicted tender" and "not predicted tender" groups are then tested for a significant difference. As a measure of error, the percentage of extra tough steaks with shear force values greater than 25 kgF placed in each category is also quoted. The results of these analyses are summarised in Table 2.18. It is arguable that category thresholds based on the characteristics of the data (e.g. > 5%, > 10% toughest etc.) are somewhat arbitrary and of little practical significance to industry. The argument is especially strong when the correlation between the reference measures (e.g. Warner-Bratzler shear force or rapid slice shear force) and consumers is not known. Price *et al.* (2008) found that a three-category scale based on consumer trials was less successful than categories based on shear force because the mean shear values of tender and intermediate categories discerned by consumers were too similar.

Table 2.15 Summary of experiments where visible-near infrared spectroscopy has been applied to predict instrumental measures of beef and lamb meat quality, showing the number and type of animals used as well as the reported coefficients of determination (R^2) and standard errors (SE) for calibration, cross-validation and prediction of meat quality parameters (further examples are given in Table 2.16 and Table 2.17).

Meat[a,b]	Number of animals with gender and genotype where available	Instrumental meat quality parameter[c] (units) and aging time where available	Calibration			Cross-validation		Prediction			RPD[d]	Reference
			n	R^2	SE	R^2	SE	*N*	R^2	SE		
Beef LT (I)	119 carcases	WBSF (kg) 69@7d & 50@14d	80	67	1.20	-	-	39	63	1.34	1.64	Park *et al.* (1998)
Beef LT (I)	70 heifers	WBSF (kg), (14d)	70	69	-	-	0.96	-	-	-	1.20	Byrne *et al*. (1998)
		WBSF (kg), (14d)	50	83	-	-	0.78	-	-	-	1.10	
		WBSF (kg), (14d)	20	89	-	-	0.67	-	-	-	1.30	
Beef LT (I)	12 Norwegian bulls	WBSF ($kg.cm^2$) (2d)	48	-	-	83	1.37	-	-	-	-	Rødbotten *et al.* (2001)
Beef LT (I)	75 steers and heifers	WBSF (N), (14d)	184	56	-	-	15.3	-	-	-	1.22	Venel *et al.* (2001)
Beef SM (I)		WBSF (N), (14d)	262	0	-	-	0	-	-	-	-	
Beef LT (I)	10 Angus heifers, 14 Hereford steers	WBSF (kgF), (2d)	22	48	1.72	-	-	-	-	-	-	Liu *et al.* (2003)
		WBSF (kgF), (range of aging)	113	49	1.57	-	-	-	-	-	-	
		L* (2d) (blooming time not given)	24	55	1.90	-	-	-	-	-	-	
		a* (2d) (blooming time not given)	24	78	1.38	-	-	-	-	-	-	
		b* (2d) (blooming time not given)	24	90	1.16	-	-	-	-	-	-	
Beef LT (I)	101 cows, 88 bulls	WBSF (N) (2d)	173	-	-	25	11.9	-	-	-	1.09	Leroy *et al.* (2004)
		WBSF (N) (8d)	172	-	-	19	8.21	-	-	-	1.16	
		L* (2d) (90 min blooming)	170	-	-	83	1.55	-	-	-	2.39	
		a* (2d) (90 min blooming)	171	-	-	39	1.15	-	-	-	1.30	
		b* (2d) (90 min blooming)	171	-	-	75	0.77	-	-	-	1.95	
		Cooking loss (%) (2d)	173	-	-	25	2.31	-	-	-	1.13	
Beef LT (I)		SSF (N) (14d)	146	38	-	-	-	146	22	-	-	Shackelford *et al.* (2005)
Lamb LT(I)	65 lambs	MIRINZ (N) Multiple measures	260	-	-	85	1.47	65	85	12.2	2.07	McGlone *et al.* (2005)
	12 lambs	MIRINZ (N) Multiple measures	48	-	-	-	1.72	260	69	-	0.97	
Beef BF (I)	120 top and bottom rounds	WBSF (lbF) range of aging, NIR system	200	41	0.98	5	1.12	-	-	-	0.96	Sawyer *et al.* (2006)
		WBSF (lbF) range of aging, ASD system	200	19	1.07	12	1.09	-	-	-	0.99	
Beef ST (I)	120 top and bottom rounds	WBSF (lbF) range of aging, NIR system	200	41	1.08	22	1.18	-	-	-	1.02	
		WBSF (lbF) range of aging, ASD system	200	45	1.06	32	1.14	-	-	-	1.05	
Beef SM (I)	120 top and bottom rounds	WBSF (lbF) range of aging, NIR system	200	85	0.59	69	0.81	-	-	-	1.44	
		WBSF (lbF) range of aging, ASD system	200	61	0.92	57	0.96	-	-	-	1.22	
Lamb LT (I)	232 Texel, Scottish blackface lambs	Intramuscular fat (%)	231	81	0.41	70	0.53	-	-	-	1.70	Andrés *et al.* (2007)
		Ultimate pH	231	26	0.15	7	0.17	-	-	-	1.00	
Beef LT (M)	148 Peidmontese bulls	WBSF ($kg.cm^2$) (8 ± 2d)	148	8	5.09	3	5.21	-	-	-	1.09	De Marchi *et al.* (2007)
		Cooking loss (%)	148	19	1.20	10	1.27	-	-	-	2.80	

[a] I = Intact, H = Homogenized, M = Minced.

[b] BF = *M. biceps femoris*, LT = *M. longissimus thoracis*, SM = *M. semimembranosus*, ST = *M. semitendinosus*.

[c] WBSF = Warner-Bratzler peak shear force, L* = Lightness, a* = Redness, b* = Yellowness, Cooking loss = The weight lost during cooking as a percentage of the un-cooked weight. [d] RPD =Ratio performance deviation (SD/SE cross-validation or prediction (where possible).

Table 2.16 Summary of further experiments (in addition to those in Table 2.15) where visible-near infrared spectroscopy has been applied to predict instrumental measures of beef and lamb meat quality, showing the number and type of animals used as well as the reported coefficients of determination (R^2) and standard errors (SE) for calibration, cross-validation and prediction of meat quality parameters (further examples are given in Table 2.17).

Meat[a,b]	Number of animals with gender and genotype where available	Instrumental meat quality parameter[c] (units) and aging time where available	Calibration			Cross-validation		Prediction			RPD[d]	Reference
			n	R^2	SE	R^2	SE	*n*	R^2	SE		
Beef LT (I)	30 Maronesa bulls	WBSF (kg/10 cm^2), 4 aging times	112	65	2.30	0.53	2.67	-	-	-	1.46	Andrés *et al.* (2008)
		Cooking loss (%)	99	85	1.00	80	1.16	-	-	-	2.65	
		L* (60 min blooming)	109	82	1.19	75	1.36	-	-	-	2.07	
		a* (60 min blooming)	100	35	1.22	29	1.28	-	-	-	0.90	
		b* (60 min blooming)	99	51	0.95	46	0.99	-	-	-	1.37	
		Sarcomere Length (µm)	30	16	0.08	2	0.10	-	-	-	0.84	
Beef LT (H)	190 young bulls	WBSF (kgF) (7d)	142	74	0.66	-	-	48	74.3	1.06	1.43	Ripoll *et al.* (2008)
Beef LT (H)	53 steers, Valles del Esla	Ultimate pH	50	41	0.06	-	0.06	-	-	-	1.17	Prieto *et al.* (2008)
		L* (60 min blooming)	50	59	1.30	-	1.50	-	-	-	1.25	
		a* (60 min blooming)	51	1	1.55	-	1.58	-	-	-	0.97	
		b* (60 min blooming)	52	35	1.44	-	1.46	-	-	-	1.16	
		Cooking loss (%)	48	14	1.57	-	1.61	-	-	-	1.04	
		WBSF (N) (7d)	49	45	9.32	-	10.00	-	-	-	1.18	
	67 young cattle	Ultimate pH (units)	61	47	0.07	-	0.08	-	-	-	1.25	
		L* (60 min blooming)	60	87	1.31	-	1.56	-	-	-	2.17	
		a* (60 min blooming)	62	71	1.03	-	1.15	-	-	-	1.57	
		b* (60 min blooming)	61	90	0.91	-	1.08	-	-	-	2.53	
		Cooking loss (%)	60	0	2.41	-	2.45	-	-	-	0.96	
		WBSF (N) (3d)	61	17	15.69	-	15.89	-	-	-	1.07	
Beef LT (I)	50 steers, 50 heifers 50 bulls	WBSF (kg cm^{-2}) (14d) fresh cut	120	25	0.45	-	-	30	6	0.23	-	Moss *et al.* (2009)
		WBSF (kg cm^{-2}) (21d) fresh cut	120	51	0.48	-	-	30	21	0.54	-	
		WBSF (kg cm^{-2}) (14d) 60 min bloomed cut	120	30	0.43	-	-	30	21	0.25	-	
		WBSF (kg cm^{-2}) (21d) 60 min bloomed cut	120	74	0.35	-	-	30	24	0.88	-	
Beef LT (I)	87 heifers	WBSF (N) (14d)	87	15	0.78	-	-	-	-	-	-	Bowling *et al.* (2009)

[a] I = Intact, H = Homogenized, M = Minced.

[b] BF = *M. biceps femoris*, LT = *M. longissimus thoracis*, SM = *M. semimembranosus*, ST = *M. semitendinosus*.

[c] WBSF = Warner-Bratzler peak shear force, L* = Lightness, a* = Redness, b* = Yellowness, Cooking loss = The weight lost during cooking as a percentage of the un-cooked weight, SSF = Slice Shear Force, WHC = Water holding capacity by the press method similar to that of Hamm (1986).

[d] RPD = Ratio performance deviation SD/SE of cross-validation or prediction (where possible).

Table 2.17 Summary of further experiments (in addition to those in Table 2.15 and Table 2.16 where visible-near infrared spectroscopy has been applied to predict instrumental measures of beef and lamb meat quality, showing the number and type of animals used as well as the reported coefficients of determination (R^2) and standard errors (SE) for calibration, cross-validation and prediction of meat quality parameters.

Meat[a,b]	Number of animals with gender and genotype where available	Instrumental meat quality parameter[c] (units) and aging time where available	Calibration			Cross-validation		Prediction			RPD[d]	Reference
			n	R^2	SE	R^2	SE	*n*	R^2	SE		
Beef LT (I)	128 steers, 66 heifers, Angus-Limousin crossbreds	L* (45 min blooming)	178	86	0.88	83	0.96	-	-	-	2.47	Prieto *et al.* (2009b)
		a* (45 min blooming)	176	86	0.71	76	0.95	-	-	-	2.02	
		b* (45 min blooming)	171	91	0.52	84	0.69	-		-	2.48	
		Slice shear force (N) (3d)	176	54	46.49	31	55.76	-	-	-	1.25	
		Slice shear force (N) (14d)	176	31	26.97	23	28.49	-	-	-	1.14	
		Volodkevitch shear force (N) (13d)	172	37	11.12	21	12.70	-	-	-	1.11	
Beef LT (I)	40 Hereford steers, multiple measurements	MIRINZ shear force (N) (un-aged)	257	71	24	-	-	124	58	28	-	Rosenvold *et al.* (2009)
		pH over time	530	85	0.19	-	-	253	83	0.20	-	
		WHC (cm^2 g^{-1}) (press method)	301	67	2.8	-	-	149	67	2.8	-	
Beef LT (I)	40 rib-eye rolls	WBSF (kgF) (14d and 28d)	40	42	0.69	31	0.73	-	-	-	1.05	Yancey *et al.* (2010)
		WBSF (kgF) (14d and 28d)	40	80	0.46	53	0.65	-	-	-	1.18	
Beef LT (M)	268 Piedmontese bulls	WBSF (kgF) (8 ± 2d)	268	21	0.48	-	-	-	-	-	-	Cecchinato *et al.* (2011)
		Cooking loss (%)	268	4	3.55	-	-	-	-	-	-	
		L* (60 min blooming)	268	64	2.12	-	-	-	-	-	-	
		a* (60 min blooming)	268	68	1.41	-	-	-	-	-	-	
		b* (60 min blooming)	268	44	1.65	-	-	-	-	-	-	

[a] I = Intact, H = Homogenized, M = Minced.
[b] LT = *M. longissimus thoracis*.
[c] WBSF = Warner-Bratzler peak shear force, L* = Lightness, a* = Redness, b* = Yellowness, Cooking loss = The weight lost during cooking as a percentage of the un-cooked weight, WHC = Water holding capacity by the press method similar to that of Hamm (1986).
[d] RPD = Ratio performance deviation SD/SE of cross-validation or prediction (where possible).

Table 2.18 Summary of the United States Meat Animal Research Centre experiments where visible-near infrared spectra from the *M. longissimus thoracis* steak surface was used to categorize the steaks into two categories (≤ or > median slice shear force [SSF] value). The number of carcases and the mean of each category for calibration and prediction datasets is provided including the percentage of samples placed in each category where the SSF was very high (> 25 kgF). The mean SSF and percentage of samples where SSF was > 25 kgF of the "not predicted tender" category were significantly higher than the "predicted tender" category in all cases (significance levels not shown).

	Calibration						Prediction						
Samples[a] and spectra	*n*	Mean	% > 25 kgF	*N*	Mean	% > 25 kgF	*n*	Mean	% > 25 kgF	*n*	Mean	% > 25 kgF	Reference
NIR Spectra collected at 2d post mortem	Predicted Tender (Predicted SSF ≤ 18.8 kgF)			Not predicted Tender (Predicted SSF > 18.8 kgF)			Predicted Tender (Predicted SSF ≤ 18.6 kgF)			Not predicted Tender (Predicted SSF > 18.6 kgF)			Shackelford *et al.* (2005)
LT SSF (kgF) (14d) USDA Select	146	16.0	5.5	146	22.0	30.1	146	16.3	5.5	146	21.6	30.1	
NIR Spectra collected at 2d post mortem							Predicted Tender (Predicted SSF ≤ 19.3 kgF)			Not predicted Tender (Predicted SSF > 19.3 kgF)			Shackelford *et al.* (2012b)[b]
LT SSF (kgF) (2d)							467	29.4	5.1 (> 40 kgF)	467	33.6	21.0 (> 40 kgF)	
LT SSF (kgF) (14d)							467	18.0	6.8	467	21.2	23.2	
NIR Spectra collected at 14d post mortem							Predicted Tender (Predicted SSF ≤ 11.8 kgF)			Not predicted Tender (Predicted SSF > 11.8 kgF)			Shackelford *et al.* (2012b)[b]
LT SSF (kgF) (14d)							467	17.7	7.3	467	21.5	22.7	
NIR Spectra collected at 14d post mortem							Predicted Tender (Predicted SSF ≤ 19.5 kgF)			Not predicted Tender (Predicted SSF > 19.5 kgF)			Shackelford *et al.* (2012b)
LT SSF (kgF) (14d) with a new model							233	17.3	5.1	233	21.9	25.0	
NIR Spectra collected at 2d post mortem	Predicted Tender (Predicted SSF ≤ 15 kgF)			Not predicted Tender (Predicted SSF > 15 kgF)			Predicted Tender (Predicted SSF ≤ 15 kgF)			Not predicted Tender (Predicted SSF > 15 kgF)			Shackelford *et al.* (2012a)
LT SSF (kgF) (14d)	554	13.9	2.0	603	16.5	7.8	503	13.8	0.8	648	16.4	8	
LT SSF (kgF) (14d)							2040	16.3	4.9	2164	19.9	21.3	
LT SSF (kgF) USDA Choice							161	16.8	2.5	52	19.2	9.6	
LT SSF (kgF) USDA Select							107	17.7	5.6	102	20.8	20.6	
LT Sarcomere Length (µm)							251	1.77	-	349	1.72	-	
LT Post Mortem Proteolysis (%)							251	42.0	-	349	34.1	-	
NIR spectra collected on LT 2d post mortem								Predicted Tender			Not predicted Tender		Shackelford *et al.* (2011)
M. semimembranosus SSF(kgF) (15d)								20.5	8.0		26.7	46	
M. gluteus medius SSF(kgF) (15d)								20.2	10		25.7	46	
M. biceps femoris SSF(kgF) (15d)								20.2	6		23.6	30	
M. adductor SSF(kgF) (15d)								17.6	2		20.0	8	

[a] LT = *M. longissimus thoracis*, SSF = Slice shear force (Shackelford *et al.* 1999a).[b] Shackelford *et al.* (2012b) initially used models they developed in Shackelford *et al.* (2005), then developed a new model based on spectra collect at 14d aging, provided medians were slightly higher in the 2012 dataset (personal communication, S.D. Shackelford, US Meat Animal Research Centre).

2.7.7 Current shortcomings of NIR spectroscopy

There are many aspects of NIR spectroscopy that require further investigation. The reviews of Prevolnik *et al.* (2004), Prieto *et al.* (2009a), and Weeranantanaphan *et al.* (2011) all concluded that a higher number of accurate reference measures are needed to develop more robust models and that this approach could eventually result in more uptake by industry. While this may be the case, it is important that these higher numbers are derived from different slaughter plants. Differences in slaughter, cutting processes and chilling are known affect meat quality. The model developed by Shackelford *et al.* (2005) was derived on samples and NIR spectra collected from two slaughter plants and later validated on samples from one of those plants (Shackelford *et al.* 2012b). Calibrating models on samples from a range slaughter plants appears to resolve this issue in terms of categorizing samples as "predicted tender" or "not predicted tender" (Shackelford *et al.* 2012a). Currently, it is not known how NIR calibration models developed on meat samples from one slaughter plant perform on samples produced at another site, so it is not known if NIR calibration models are plant specific. If NIR calibration equations are indeed plant specific, plant specific (or cross-plant) calibration equations will be required and a plant specific validation trial would also be required which may be problematic for meat processors and industry. Further work is also required to address the relationship between muscles and it has yet to be determined if NIR predictions on the loin are robust enough to be used on other cuts of meat. Sawyer *et al.* (2006) successfully predicted tenderness on *M. semimembranosus*, *M. semitendinosus* and *M. biceps femoris* from NIR scans taken on those respective muscles (Table 2.15), but *M. longissimus thoracis* was not included in their experiment so a comparison between these muscles and *M. longissimus thoracis* predictions is not possible. Several reports have shown that peak shear force of *M. Longissimus thoracis* is generally not highly correlated to peak shear force in other muscles, with correlation coefficients mostly lower than 0.5 (Shackelford *et al.* 1995; Johnston *et al.* 2003; Rhee *et al.* 2004). One report involving 75 animals (Venel *et al.* 2001) and investigated NIR on both *M. longissimus thoracis* and *M. semimembranosus* reported that NIR was unable to predict the organoleptic properties of the latter despite being able to predict Warner-Bratzler shear force on the former with an R^2 of 51%. Certainly, there is a theory in the MSA system that an individual muscle-based model is more appropriate than a single muscle model based on the loin (Polkinghorne and Thompson 2010).

The field of view of the NIR measurement head varies between models of NIR spectrometer, a larger measurement head, has a larger field of view. The placement of the probe is important since this can affect the level of fat or lean present in the field of view. These factors are likely to influence the NIR spectra and open up the potential for operator error, to date, few studies have addressed this important area. Operator error may also be a problem with regard to the blooming time of meat, particularly if the blooming time is varied before recording NIR spectra (Moss *et al.* 2010). Shackelford *et al.* (2012a) solved this problem by developing prediction equations based on a range of blooming times. Hyperspectral imaging may be a solution to the probe placement part of operator error, since in this technology, NIR spectra are collected for every pixel in the image (Naganathan *et al.* 2008; Cluff *et al.* 2008; Wu *et al.* 2010). A region of interest can be selected from the image and NIR spectra analysed from a selected tissue type (Naganathan *et al.* 2008).

2.7.8 Further directions for NIR spectroscopy

To date, the majority of NIR predictions on whole meat quality have been on a relatively small number beef and pork, there is far less information available on the predictive ability of NIR in other species of red meat, particularly lamb and venison. In lamb, there are only two published articles to date that have reported some evidence that NIR spectroscopy can be used to predict lamb meat quality (McGlone *et al.* 2005; Andrés *et al.* 2007). There are currently no reports of NIR being used to predict venison quality attributes. There is a clear need for more investigation of NIR in both lamb and venison. The reviews of Prevolnik *et al.* (2004); Prieto *et al.* (2009a) and Weeranantanaphan *et al.* (2011) detail the developments in NIR up to 2010. The time span of these reviews allows some comparison to be made with regard to the development and uptake of NIR technology in the meat industry. In terms of development, all three reviews conclude that the ability of NIR spectroscopy to predict sensory and technological aspects of meat quality is rather poor. The main reason cited for this is the poor precision of the reference methods of meat quality against which NIR calibration equations are made. As a result Prevolnik *et al.* (2004) reported there had been poor uptake by industry, a sentiment which appears not to have changed in recent years (Weeranantanaphan *et al.* 2011). Prieto *et al.* (2009a) speculated that the

uptake of NIR by industry would improve as error in reference measures and prediction calibrations is reduced through the use of a larger, more heterogeneous group of samples.

2.8 Summary and conclusions for NIR

The main conclusions regarding the use of NIR to predict meat quality characteristics from the current review are as follows:

1. The accuracy and repeatability of NIR spectroscopy for predicting meat quality traits is highly variable and has been generally been assessed on small numbers of animals.

2. The prediction accuracy of NIR spectroscopy for predicting meat quality traits is highly dependant on accurate and repeatable reference measures. Many meat quality reference measures such as sensory scores and shear force traits are not highly repeatable, and this has been cited on several occasions as the reason for the poor prediction accuracy of NIR spectroscopy.

3. By considering tenderness in categories, some researchers have been able to improve the apparent effectiveness of NIR spectroscopy by removing the need to account for variation within the categories. Unfortunately very few such reports have based the tenderness category thresholds on consumer or trained sensory panel data or relevance to the meat industry requirements, so results must be interpreted in light of this fact.

4. Many researchers have reported the performance of an NIR calibration model by internal cross-validation which uses the same samples as were used in the calibration phase. It is arguable that prediction on an independent dataset would be a more appropriate validation method.

5. It is not known if NIR calibration models are site-specific, but it is possible to develop calibrations from data collected across different processing plants.

6. The extent to which operator error can affect NIR prediction is not known.

7. It is not known whether NIR calibration models developed on one muscle can be applied to other muscles with similar accuracy and repeatability.

8. There are very few published reports where NIR has been applied to predict lamb and venison meat quality traits.

9. Hyperspectral imaging appears to offer some advantages over current NIR spectroscopy by enabling spectra from a region of interest to be extracted and used in a calibration equation.

Rather than a list of NIR shortcomings, the conclusions points above should be viewed as areas that need further investigation by researchers. The ability to predict meat eating quality and to factor this into carcase evaluation systems is summarised by the following quote from Gernert *et al.* (2005) "only when producers can translate consumer wishes into physical product characteristics, and only when consumers can then infer desired qualities from the way the product has been built, will quality be a competitive parameter for food producers".

3 Prediction of saleable meat yield from the sirloin and fillet cuts of beef carcases from different breeds and genders using video image analysis (VIA) and classification information

Presentations based on the results reported in this chapter:

Craigie CR, Ross DW, Maltin CA, Nath M, Hoskin SO, Morris ST, Roehe R (2009) Objective measures of predicted sirloin and fillet weights from commercial cattle - preliminary findings. *Proceedings of the 55th International Congress of Meat Science and Technology, Copenhagen, Denmark* 503-506.

Craigie CR, Purchas RW, Maltin CA, Bunger L, Hoskin SO, Ross DW, Morris ST, Roehe R (2010) Video image analysis and near infrared spectroscopy applied to beef carcass evaluation. *Institute of Veterinary, Animal and Biomedical Sciences Research Colloquium, Palmerston North, 25th November 2010*.

Craigie CR, Ross DW, Maltin CA, Purchas RW, Morris ST, Roehe R (2010) The relationship between beef quality and carcass quality attributes measured under commercial conditions. *Proceedings of the British Society of Animal Science annual conference, Belfast* Abstract 129.

Abstract

Carcase quality of 72 steers, 48 heifers and 21 bulls from continental and dairy crossbred genotypes were compared on the basis of conformation, fatness and saleable meat yield (SMY%) of the fillet and trimmed boneless sirloin cuts. Comparisons between genotype-gender groups showed that steers from beef breeds had higher EUROP conformation scores than those from dairy crossbreds which corresponded to a higher SMY% of sirloin and fillet. The EUROP grid underestimated the sirloin SMY% of Charolais heifers due to their higher muscle-to-bone ratio. Furthermore, the 141 carcases were used to assess the accuracy with which video image analysis (VIA) and visual classification in a commercial abattoir predicted the weight and SMY% of the sirloin and fillet. Both VIA and the visual carcase classification systems resulted in similar accuracies for prediction of sirloin SMY% (R^2 = 58%, RSD = 0.35% for VIA and R^2 = 57%, RSD = 0.35% for visual classification respectively) but fillet yield was poorly predicted by both VIA and visual classification systems. Including the weight of excess fat removed during sirloin trimming as an additional covariate for sirloin SMY%

prediction did not offer any substantial improvement in predictive ability. Inclusion of bone weight did show some promise for improving the prediction accuracy of fillet SMY%.

3.1 Introduction

Within the European Union, adult bovine carcases are evaluated according to the EUROP carcase classification scheme under the European Community regulations 1208/81 and 2930/81(European Community 1981a; European Community 1981b). The EUROP scheme includes carcase conformation scores on a 15-point scale with 5 main classes, E, U, R, O and P, and 10 sub-classes, and five main fatness scores (1, 2, 3, 4 and 5) also with 10 sub-classes (Fisher 2007). During development of the EUROP scheme, no attempt was made to relate it to the amount of lean meat as a percentage of the dressed carcase, known as saleable meat yield (SMY%), which is lean meat sold with a certain amount of fat still attached, (Kempster *et al.* 1980). The reason for the lack of alignment between EUROP and yield traits was because there was no standardized definition of SMY% (Allen 2003). It has been argued that carcase evaluation must relate to SMY% so prices can give clear production objectives to producers through a value-based marketing system (Cross and Whittaker 1992).

As a result, recent research has focused on relating carcase classification to SMY% (Conroy *et al.* 2009; Conroy *et al.* 2010a; Conroy *et al.* 2010b). The EUROP scheme for carcase evaluation has been widely criticized on account of its subjective (visual) mode, even when assessed by a trained classifier using photographic references. Although there is little evidence to support this criticism, it is impossible to demonstrate the objectivity of the system as long as human classifiers are involved (Allen 2003). To address this weakness, objective carcase evaluation methods based on video image analysis (VIA) were developed which allowed a large number of variables (lengths, widths, areas, volumes etc.) to be measured on a carcase in a matter of seconds. VIA systems that assess whole sides of beef can be installed on-line in abattoirs to operate autonomously at line speeds up to 800 beef carcases per hour (Ross *et al.* 2011) and up to 800 lamb carcases per hour (Rius-Vilarrasa *et al.* 2009).

Four different makes of commercially available whole-side VIA systems have been used in Europe to classify beef carcases according to the EUROP conformation and fat class grid, including the BCC-2 (Borggaard *et al.* 1996), VIAscan (Allen and Finnerty 2001) VBS 2000 (Augustini *et al.* 1997) and the Normaclass Machine à classer (MAC) (Allen 2007). Features of these VIA systems are reviewed in Section 2.4.6.

In an experiment undertaken by Teagasc in the Republic of Ireland, the VIAscan, VBS 2000 and BCC-2 systems were able to predict SMY% from the same carcases with similar accuracies (RSD between 1.1 and 1.2%; (Allen and Finnerty 2001). VIA has since been successfully applied to carcase classification in the Republic of Ireland on an industrial scale since 2004 using VBS 2000 machines (Allen 2007; Pabiou *et al.* 2011b). Many studies have investigated the ability of the VBS 2000 to assess carcases from a variety of cattle populations in Germany, (Augustini *et al.* 1997; Branscheid *et al.* 1998; Sonnichsen *et al.* 2006; Brinkmann 2007), Norway (Jørgenvåg *et al.* 2009) and the Republic of Ireland (Allen and Finnerty 2000; Allen and Finnerty 2001; Allen 2005; Pabiou *et al.* 2009; Pabiou *et al.* 2011b). Most of these experiments have assessed the ability of VIA to classify carcases according to the EUROP grid, yet all whole-side VIA systems are able to directly predict carcase SMY%, this feature has seldom been assessed under commercial conditions.

The aims of the current experiment were:

- To compare carcase traits and the trimmed, boneless yield of sirloin and fillet meat for groups of cattle differing in gender and breed.
- To assess the accuracy with which these characteristics were predicted by VIA and visual carcase classification systems.

3.2 Material and methods

3.2.1 Animals

Between March and May 2009, 141 cattle below 30 months of age were selected for inclusion in the experiment at the point of inspection and classification in a commercial abattoir located in Perthshire, Scotland. Each week for 6 weeks, 4 steers and 4 heifers were selected from Charolais and Limousin breeds and 4 bulls and 4 steers were

selected from dairy breeds based on passport breed codes, age, and gender. After 6 weeks the data set comprised 24 Charolais heifers (CH), 23 Charolais steers (CS), 25 Limousin heifers (LH), 24 Limousin steers (LS), 24 dairy steers (DS) and 21 dairy bulls (DB). Breed codes are entered on the passports by the producer and are derived from the sire breed (Todd *et al.* 2011). All Charolais and Limousin were crossbreds, whereas of the 45 dairy animals, 34 were Holstein-Friesians, 4 were Holstein Friesian crosses, 4 were Holsteins and 3 were British Friesians according to the breed code descriptions (Todd *et al.* 2011).

3.2.2 Abattoir protocol

Cattle were stunned using a captive bolt pistol, exsanguinated, and subjected to electrical stimulation (90 volts for 30 seconds at 10 minutes post mortem) during hide removal. Carcases were dressed to UK specification as described in the Meat and Livestock Commercial Services Limited beef authentication manual (www.mlcsl.co.uk). Visual carcase classification for conformation and fatness was performed by a trained Meat and Livestock Commercial (MLC) services human assessor using the more-restricted version of EUROP classification scale used in the UK (MLC_{uk}) that uses 8 of the 15 categories for conformation ($MLCC_{uk}$) and 7 out of the 15 categories for fatness ($MLCF_{uk}$) (European Community 1981a; European Community 1981b). Both $MLCC_{uk}$ and $MLCF_{uk}$ values were expressed on the full 15 point scale for analysis (the conversions from UK scale to the 15 point scale are provided in Table 3.2). A VIA system (VBS 2000, E+V GmbH, Germany) also estimated classification scores for conformation and fatness on both the UK ($VIAC_{uk}$ and $VIAF_{uk}$, respectively, or VIA_{uk} collectively) and full 15 point scales ($VIAC_{15}$ and $VIAF_{15,}$ respectively or VIA_{15} collectively) that are common across the European Union (De Boer *et al.* 1974). A direct prediction of whole-carcase SMY% was also obtained from the VIA system (VIA-SMY%). VIA data was available on 137 out of 141 carcases. The VIA system was operated on-line and independently from the human classifier. Hot carcase weight (HCW) was recorded at the same point as carcase classification and was used as one input to the VIA system.

3.2.3 Carcase cutting protocol

All 141 carcases were quartered between the 10th and 11th ribs at 48 hours post mortem into hind (pistola) and forequarters. A schematic of the cutting protocol is shown in Figure 3.1. The complete sirloin (CSL) was removed bone-in and untrimmed from the hindquarter as described by Kempster *et al.* (1980). The untrimmed fillet (FIL) (containing *M. Psoas major* and *M. Psoas minor*) was removed from the complete sirloin and weighed, the bone (BON) and excess fat (XSF) (fat trimmed to a maximum depth of 9 mm at the ¾ point across the *longissimus thoracis et lumborum* muscle at the 10th rib) of the sirloin were also weighed. The resulting trimmed, boneless saleable meat of the sirloin (SS), which included parts of *M. longissimus thoracis et lumborum*, *M. multifidus dorsi* and *M. longissimus costarum,* was weighed, and the yield of saleable sirloin [SS/HSW (%)] and fillet [FIL/HSW (%)] were calculated as a percentage of the hot side weight (0.5 x the HCW), while sirloin muscle-to-bone ratio (M:B) was calculated as SS divided by BON.

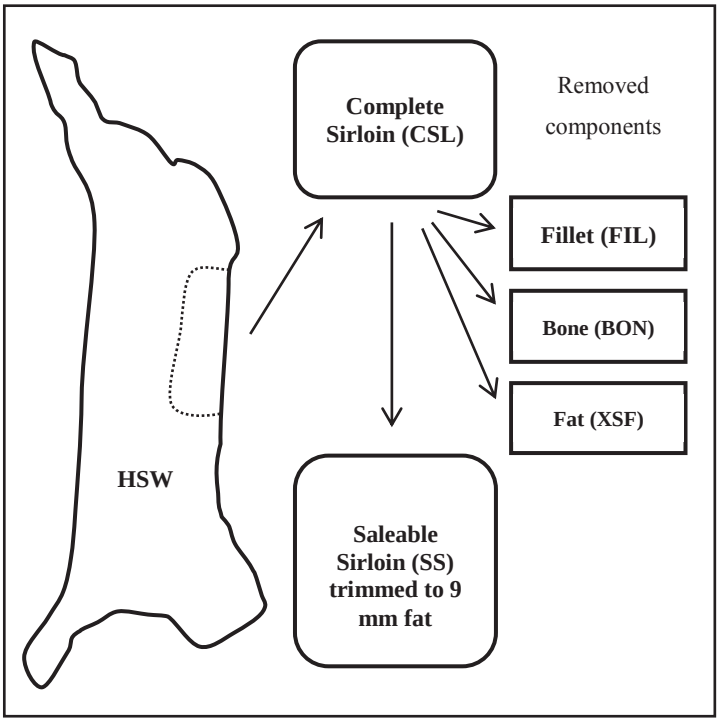

Figure 3.1 Schematic of the carcass cutting procedure.

3.2.4 Statistical analysis

The percentage distribution of carcases by conformation and fat classification as assigned by the human classifier was performed using the FREQ procedure of SAS (SAS Inst. Inc., Cary, NC). Traits analyzed included hot carcase weight, age at slaughter, carcase traits assessed by visual and VIA carcase classification, weights of the sirloin and its dissected components, and yields of the latter. A general linear model (PROC GLM) was used to estimate least-squares (L-S) means for the genotype-gender effects (CH, CS, LH, LS, DS and DB) after adjusting for batch (determined by slaughter day, $n = 6$). Additionally, HCW was fitted as a covariate for all traits except for animal age. Comparison of L-S means among genotype-gender effects were performed using t-tests (Bonferroni adjusted to account for multiple comparisons). Moreover, three non-orthogonal contrasts were employed to make comparisons between genotypes and genders using "estimate" statements in SAS. Then, pair-wise residual correlations among traits were estimated using the MANOVA option in PROC GLM, in the first analysis adjusting for batch effects only, and in the second analysis by additionally adjusting for genotype-gender effects. Finally, several different general linear models were fitted to evaluate the accuracy of those models (R^2 and RSD) to predict sirloin and fillet cut weights, cut yields and sirloin M:B ratios.

Table 3.1 A list of the six genotype-gender groups and corresponding abbreviations.

Genotype-gender group	Abbreviation
Charolais cross heifer	CH
Charolais cross steer	CS
Limousin cross heifer	LH
Limousin cross steer	LS
Dairy cross steer	DS
Dairy cross young bull	DB

3.3 Results and discussion

The distribution of the experimental carcases into EUROP conformation and fat classes (as allocated by a trained assessor on the UK scale) is presented in Table 3.2, together with the distribution of prime cattle slaughtered in Great Britain (GB) in 2009 for comparison. Most carcases in this study were in the in the R (45.5%) –O (22.8%) and

O+ (14.9%) conformation classes and the 4L (44.0%), 4H (30.5%) and 3 (21.3%) fatness categories. The sample distribution was broadly similar to that in the GB prime cattle population slaughtered in 2009, but there was a higher proportion of 4H carcases (30.5% vs. 12.1% in the GB population), fewer in the O+ and more in the –O conformation classes in the sample set. These differences are probably a result of the inclusion of the DB, which tended to have a poorer than average conformation and the fact that some abattoirs were not penalizing fatter carcases at that time the experiment was conducted.

Table 3.2 The distribution (percentage) of carcases (*n* = 141) used in the current experiment based on visually-assigned EUROP conformation and fat classes. The distribution (%) of all prime beef animals slaughtered in Great Britain in 2009 is included for comparison.

	Fatness[a]							
	2 (5)	3 (8)	4L (10)	4H (12)	5L (13)	5H (15)	Total	GB 2009 (%)[c]
Conformation[a]								
U+ (12)[b]	0	0	0.7	1.4	0.4	0	2.5	2.3
–U (10)	0	1.4	6.4	5.0	0	0	12.8	13.0
R (8)	0.7	4.3	20.6	19.2	0.7	0	45.5	44.2
O+ (6)	0	4.3	8.5	1.4	0.7	0	14.9	26.6
–O (4)	1.4	11.4	7.8	3.6	0	0	22.8	11.5
P+ (3)	0.4	0.9	0	0	0	0	1.3	1.8
–P (1)	0.7	0	0	0	0	0	0.7	0.3
Total	1.1	21.3	44.0	30.5	1.42	0	100	
GB 2009 (%)[c]	10.8	30.8	44.0	12.1	0.9	0.1		

[a] Conformation and fatness classification scores determined by a trained assessor on the UK scale.
[b] Numbers in brackets are the corresponding categories on the 15-point EUROP scale (Fisher 2007).
[c] GB 2009 refers to all prime cattle slaughtered in England, Scotland and Wales between 1st January and 31st December 2009 (Courtesy of Kim Matthews, English Beef and Lamb Executive).

3.3.1 Genotype-gender effects

Least-squares means of genotype-gender effects for carcase classification (all on a scale of 1-15) are shown in Table 3.3. Significant batch effects were present for $VIAF_{uk}$, $VIAF_{15}$ and VIA-SMY% (data not shown). As might be expected, beef steers (LS and CS) had significantly higher conformation class scores than dairy crossbred steers (DS), with this being more pronounced with visual classification, than $VIAC_{uk}$, or $VIAC_{15}$ ($P < 0.001$ in all cases). There was no significant difference between steers for fatness, but DS were significantly fatter than DB according to both visual and VIA classifications ($P < 0.001$) and the difference was more pronounced for the $VIAF_{15}$ system (3.29 ± 0.44, $P < 0.001$). In contrast, there was no significant difference in conformation class between DS and DB according to $MLCC_{uk}$, whereas $VIAC_{uk}$ and $VIAC_{15}$ determined that DB had higher conformation scores than DS with differences of 0.92 ± 0.39 ($P = 0.02$) and 0.97 ± 0.36 ($P < 0.008$) respectively. Overall, heifers were significantly fatter than

steers (3rd contrast in Table 3.3) with the largest difference being found when assessed by $MLCF_{uk}$ (1.75 ± 0.36, $P < 0.001$). This difference was considerably less when assessed by $VIAF_{uk}$ (1.59 ± 0.40, $P = 0.001$) and VIA_{15} (1.49 ± 0.39, $P < 0.001$). Heifers were found to have higher conformation scores than steers, but only when assessed by $VIAC_{uk}$ (0.88 ± 0.35, $P = 0.01$), this difference was no longer significant based on $VIAC_{15}$ (0.56 ± 0.32, $P = 0.08$).

Carcase conformation and fatness as determined visually and by VIA were considerably different. $MLCF_{uk}$ scores were consistently lower than $VIAF_{15}$ and $VIAF_{uk}$ in both beef genotypes, yet $VIAF_{uk}$ predicted the fat class of DB to be approximately a sub-class (1/15) lower than values determined by $MLCF_{uk}$. In the comparison between DS and DB, the $VIAF_{15}$ estimated fatness difference between the genders were higher in DS by around 3 sub-classes whereas $MLCF_{uk}$ estimated the difference to be around 1.5 sub-classes. In a previous report where dairy crossbred bulls and steers were assessed by VIA in the Republic of Ireland on the 15-point EUROP scale, the difference in mean fat-class between genders was 0.7 sub-classes (Conroy *et al.* 2010b), but of the prime cattle slaughtered in the UK in 2009 (1.95 million head), the fat class of young bulls was 2-3 sub-classes (visually assessed on the UK scale but converted to the 15 point scale) lower than steers and heifers (personal communication, Kim Matthews, English Beef and Lamb Executive).

The difference between genotype-gender effects is important (particularly in terms of SMY%), but the ability of visual and VIA carcase evaluation systems to detect these differences is more appropriate basis of comparison. Further investigation into the significance of the differences between VIA and visual classification need to include carcase classification as a whole, i.e. fat class and conformation class since both are used together to describe the merits of a carcase.

Table 3.3 Least-squares means for genotype-sex groups adjusted for hot carcase weight and batch effects as well as contrasts between genotype-gender groups.

Trait (Abbreviation)	Least-squares means of genotype-gender groups[11]						Contrast			Effects[12]		R^2 [%] (RSD)[13]
	CH	CS	LH	LS	DS	DB	Beef steers vs. Dairy steers	Dairy steers vs. Dairy bulls	Beef steers vs. Beef heifers	HCW	Group	
	$n = 24$	$n = 23$	$n = 25$	$n = 24$	$n = 24$	$n = 21$						
Hot carcase weight (HCW) (kg)	318.8^{a}	400.6^{b}	301.2^{a}	351.1^{b}	322.5^{a}	303.7^{a}	< 0.001	0.06	< 0.001	-	< 0.001	52.2 (34.55)
Age at slaughter (Age) (d)	753.8^{a}	704.0^{a}	741.2^{a}	709.6^{a}	756.3^{a}	477.5	0.03	< 0.001	0.03	-	< 0.001	56.3 (88.70)
$MLCF_{uk}$[1]	11.50^{a}	9.80^{bcd}	11.19^{ab}	9.38^{cd}	9.96^{c}	8.43^{d}	0.35	< 0.001	< 0.001	0.004	< 0.001	40.8 (1.37)
$MLCC_{uk}$[2]	8.13^{b}	8.29^{b}	8.07^{b}	8.15^{b}	4.73^{a}	4.47^{a}	< 0.001	0.41	0.66	< 0.001	< 0.001	80.1 (1.01)
Video Image Analysis												
$VIAF_{uk}$[3]	11.94^{a}	10.54^{ab}	11.63^{a}	9.85^{b}	9.84^{b}	7.06^{c}	0.42	< 0.001	< 0.001	0.24	< 0.001	59.6 (1.51)
$VIAF_{15}$[4]	11.70^{a}	10.54^{ab}	12.01^{a}	10.00^{b}	10.44^{b}	7.62^{c}	0.64	< 0.001	< 0.001	0.23	< 0.001	62.5 (1.46)
$VIAC_{uk}$[5]	7.83^{b}	7.14^{b}	7.83^{b}	6.76^{b}	4.35^{a}	5.26^{a}	< 0.001	0.02	0.01	< 0.001	< 0.001	63.8 (1.30)
$VIAC_{15}$[6]	7.65^{b}	7.26^{b}	7.64^{b}	6.92^{b}	4.65^{a}	5.62^{a}	< 0.001	0.008	0.08	< 0.001	< 0.001	65.8 (1.18)
VIA-SMY (%)[7]	77.39^{c}	76.78^{ac}	77.26^{c}	77.09^{c}	75.23^{b}	75.84^{ab}	< 0.001	0.03	0.13	< 0.001	< 0.001	63.1 (0.94)
Weights												
Saleable sirloin (SS) (kg)	7.73^{b}	7.21^{ab}	7.65^{b}	7.26^{ab}	6.79^{a}	6.72^{a}	0.01	0.69	0.005	< 0.001	< 0.001	81.8 (0.61)
Fillet (FIL) (kg)	3.56^{c}	3.56^{bc}	3.58^{c}	3.51^{bc}	3.23^{ab}	3.15^{a}	0.001	0.12	0.70	< 0.001	< 0.001	79.3 (0.27)
Excess fat (XSF) (kg)	1.21^{b}	1.10^{ab}	1.21^{b}	1.11^{b}	1.18^{b}	0.77^{a}	0.38	< 0.001	0.19	< 0.001	< 0.001	47.6 (0.30)
Bone (BON) (kg)	3.53^{bc}	3.56^{bc}	3.41^{bc}	3.47^{bc}	3.61^{bc}	3.85^{a}	0.38	0.05	0.70	< 0.001	0.008	53.7 (0.40)
Yields												
SS/HSW (%)[8]	4.67^{b}	4.32^{ab}	4.62^{b}	4.36^{ab}	4.06^{a}	4.02^{a}	0.08	0.67	0.002	0.23	< 0.001	53.7 (0.36)
FIL/HSW (%)[9]	2.14^{d}	2.14^{cd}	2.16^{bd}	2.11^{cd}	1.97^{ac}	1.88^{ab}	0.001	0.07	0.51	0.09	< 0.001	32.4 (0.16)
M:B[10]	2.22^{b}	2.04^{ab}	2.26^{b}	2.10^{ab}	1.98^{ab}	1.73^{a}	0.43	0.05	0.14	0.02	< 0.001	23.4 (0.42)

Note: For VIA traits the following numbers apply: CS ($n = 22$), LH ($n = 24$) and LS ($n = 22$). Least-squares means within a row sharing a common superscript letter are not significantly ($P \leq 0.05$) different.

[1] $MLCF_{uk}$ = Meat and livestock commission fatness (operating on the UK scale, but expressed on 15-point scale).

[2] $MLCC_{uk}$= Meat and livestock commission conformation (operating on the UK scale, but expressed on 15-point scale).

[3] $VIAF_{uk}$ = Video image analysis fatness (operating on the UK scale, but expressed on 15-point scale).

[4] $VIAF_{15}$ = Video image analysis fatness operating on the 15-point scale.

[5] $VIAC_{uk}$ = Video image analysis conformation (operating on the UK scale, but expressed on 15-point scale).

[6] $VIAC_{15}$ = Video image analysis conformation operating on the15-point scale.

[7] VIA-SMY(%) = Video image analysis prediction of total carcase saleable meat yield.

[8] SS/HSW(%) = Yield of saleable sirloin meat (boneless with fat trimmed to 9 mm) as a percentage of hot side weight.

[9] FIL/HSW(%) = Yield of fillet as a percentage of hot side weight.

[10] M:B = Muscle-to-bone ratio of the sirloin (excluding the fillet).

[11] CH = Charolais heifers, CS = Charolais steers, DS = Dairy steers, DB = Dairy bulls LH = Limousin heifers and LS = Limousin steers. Pairwise comparisons between the means are provided for information purposes, contrasts are used to draw specific comparisons between the genotype-gender groups.

[12] Effects (P values) HCW = Hot carcase weight (included as a covariate), Group = genotype-sex group (included as a fixed effect, $n = 6$) Batch was included as a fixed effect ($n = 6$) for all traits, (data not shown).

[13] R^2 = Coefficient of determination, (RSD = Residual standard deviation).

3.3.2 Carcase classification

Further enquiry into the ability of the EUROP carcase classification to determine SMY% is only possible when either fat class or conformation class (but not both) are significantly different in a given comparison at a common level of fat trimming (Kempster 1986). The present results satisfy this condition in the first contrast in Table 3.3: "Beef steers vs. Dairy steers" and between genders, within the Charolais genotype only (data not shown). In the first contrast, the beef steers had significantly higher conformation scores than the dairy steers according to $MLCC_{uk}$, $VIAC_{uk}$ and $VIAC_{15}$, but there were no significant differences in fat class between the genotypes. In Charolais, CH had higher fat class scores than CS according to $MLCF_{uk}$, $VIAF_{uk}$ and $VIAF_{15}$ but there was no significant difference in carcase conformation. SMY% traits SS/HSW (%) and FIL/HSW (%) were both significantly greater in the beef steers than DS ($P = 0.02$ and $P = 0.001$ respectively) as was the conformation score ($P < 0.001$). Within the Charolais genotypes, CH had significantly higher fatness scores than steers (CS) and showed significantly greater yields of SS/HSW (%) ($P = 0.005$). No significant difference in FIL/HSW (%) ($P = 0.95$) were detected between CH and CS.

The inconsistent relationship between EUROP classification and SMY% in the current results may have several causes; including over fat carcases (Purchas and Wilkin 1995), or may be due to an inconsistent relationship between muscularity and M:B, particularly in the case of heifers, where the M:B may be higher than that of bulls and steers (Purchas *et al.* 2002b). The inconsistencies between genders within genotypes are not surprising, given that a meta-analysis on 903 carcases in 11 experiments failed to find a significant relationship between carcase conformation class and muscle proportion (Keane *et al.* 2000). On the other hand, Keane *et al.* (2000) reported fat class was negatively associated with both muscle and bone proportions in seven of the 11 experiments. The inconsistency between carcase shape and SMY% has also been found in lambs where prediction of lean meat yield based on carcase shape, will be underestimated in females and overestimated in males (Johnson *et al.* 2005). Upon further investigation in the current analysis, CH did have a higher M:B ratio than CS but the differences were not statistically significant ($P = 0.24$). More importantly, the differences in M:B may be biased if carcase shape is used as a predictor of M:B (Purchas *et al.* 2002b) and ultimately SMY%. Further investigation into the relationship

between carcase conformation and fatness and SMY% is possible by comparing the correlations between the traits. Table 3.4 shows correlations (adjusted for batch only, as well as adjusted for batch and genotype-gender effects) between HCW, carcase classification and SS/HSW (%), FIL/HSW (%) and M:B ratio.

After adjusting for batch effects, $MLCF_{uk}$ was positively correlated to HCW (r = 0.2), and carcase conformation was positively correlated to HCW in all cases (r = 0.47-0.55) (Table 3.4). Both fatness and conformation were moderately positively correlated to SS/HSW (%). Conformation classes had the closest positive correlations to SS/HSW (%) in the first instance (r = 0.49-0.52) with little difference between VIA (UK and 15-point scale) and $MLCC_{uk}$, but after adjusting for the genotype-gender effects, the correlation was greatly reduced (r = 0.28-0.31). This is especially evident in the FIL/HSW (%) where conformation and fatness were not correlated to FIL/HSW (%) after the genotype-gender effects were removed. M:B was positively correlated to HCW, but as expected M:B and fatness were not correlated after adjustment for genotype-gender effects which is probably due to the lower M:B in the DB genotype-gender group (Table 3.3). Conformation correlated to M:B but large improvements were seen when no adjustments were made for genotype-gender effects (Table 3.4). This is most likely because M:B varies by gender and genotype.

In a previous experiment (Kempster and Harrington 1980), the correlation between carcase conformation and the percentage of high-priced cuts (as a percentage of total saleable meat weight rather than total side weight) was similarly low, although only six classification categories were used. The highest correlations between sirloin SMY% and composition in the current results were obtained between VIA operating on the 15 point scales, which suggests the scale is appropriate for the UK prime cattle population. The correlations between yield and classification should be interpreted with caution because a positive relationship between carcase fatness and SS/HSW (%) was observed in CH, and $MLCF_{uk}$ was positively correlated to SS/HSW (%) across all genotype-gender groups. Furthermore, it is also possible that the total carcase SMY% may not have the same relationship to conformation as was presented for the sirloin region in the current results.

After adjusting for genotype-gender effects and batch, in the current analysis, there were no significant correlations between measures of conformation and fat class, which corroborates previous findings (Drennan *et al.* 2007; Drennan *et al.* 2008; Conroy *et al.* 2009; Conroy *et al.* 2010a; Conroy *et al.* 2010b) who found fatness and conformation were not significantly correlated in the absence of genotype and gender effects. There is further agreement between the current results and those of Kempster and Harrington (1980) who concluded that breed generally provided a more precise prediction of carcase composition than carcase conformation in steers.

Table 3.4 Residual correlations and *P* values (where significant) adjusted for batch [B] and residual correlations after adjusting for both B and genotype-gender group [G] between carcase classification and yield traits (percentage of hot side weight) and loin region muscle-to-bone ratio for 137 carcases.

Trait	HCW[a]		SS/HSW (%)[h]		FIL/HSW (%)[i]		M:B[j]	
Effects	B	B+G	B	B+G	B	B+G	B	B+G
HCW[a]	-	-	0.09	0.11	–0.03	–0.16	0.28 (0.001)	0.23 (0.01)
$MLCF_{uk}$[b]	0.20 (0.02)	0.28 (0.002)	0.41 (< 0.001)	0.18 (0.05)	0.16	–0.16	0.28 (0.001)	0.10
$VIAF_{uk}$[c]	0.16	0.10	0.38 (< 0.001)	0.10	0.27 (0.002)	–0.16	0.29 (< 0.001)	0.01
$VIAF_{15}$[d]	0.16	0.11	0.42 (< 0.001)	0.02	0.27 (0.002)	–0.17	0.30 (< 0.001)	0.02
$MLCC_{uk}$[e]	0.54 (< 0.001)	0.47 (< 0.001)	0.52 (< 0.001)	0.30 (< 0.001)	0.44 (< 0.001)	0.12	0.44 (< 0.001)	0.28 (0.002)
$VIAC_{uk}$[f]	0.47 (< 0.001)	0.42 (< 0.001)	0.51 (< 0.001)	0.31 (< 0.001)	0.32 (< 0.001)	0.00	0.40 (< 0.001)	0.25 (0.005)
$VIAC_{15}$[g]	0.55 (< 0.001)	0.49 (< 0.001)	0.49 (< 0.001)	0.28 (0.001)	0.32 (< 0.001)	0.04	0.45 (< 0.001)	0.34 (< 0.001)

[a] HCW = Hot carcase weight.
[b] $MLCF_{uk}$ = Meat and livestock commission fatness (operating on the UK scale, but expressed on 15-point scale).
[c] $VIAF_{uk}$ = Video image analysis fatness (operating on the UK scale, but expressed on 15-point scale).
[d] $VIAF_{15}$ Video image analysis fatness operating on the 15-point scale.
[e] $MLCC_{uk}$= Meat and livestock commission conformation (operating on the UK scale, but expressed on 15-point scale).
[f] $VIAC_{uk}$ = Video image analysis conformation (operating on the UK scale, but expressed on 15-point scale).
[g] $VIAC_{15}$ = Video image analysis conformation (15-point scale).
[h] SS/HSW (%) = Yield of saleable sirloin meat (boneless with fat trimmed to 9 mm) as a percentage of hot side weight.
[i] FIL/HSW (%) = Yield of fillet as a percentage of hot side weight.
[j] M:B = Muscle-to-bone ratio of the sirloin (excluding the fillet).

Table 3.5 Residual correlations and *P* values (where significant) adjusted for batch [B] and residual correlations after adjusting for both B and group [G] between VIA-predicted and visually assessed carcase classification methods on 137 carcases.

Trait	$MLCF_{uk}$[a]		$VIAF_{uk}$[b]		$VIAF_{15}$[c]		$MLCC_{uk}$[d]		$VIAC_{uk}$[e]	
Effects	B	B+G	B	B+G	B	B+G	B	B+G	B	B+G
$VIAF_{uk}$[b]	0.78 (< 0.001)	0.65 (< 0.001)	1	1	-	-	-	-	-	-
$VIAF_{15}$[c]	0.79 (< 0.001)	0.69 (< 0.001)	0.96 (< 0.001)	0.92 (< 0.001)	1	1	-	-	-	-
$MLCC_{uk}$[d]	0.39 (< 0.001)	0.16	0.47 (< 0.001)	0.01	0.49 (< 0.001)	0.07	1	-	-	-
$VIAC_{uk}$[e]	0.37 (< 0.001)	0.15	0.38 (< 0.001)	–0.01	0.38 (< 0.001)	0.00	0.79 (< 0.001)	0.50 (< 0.001)	1	-
$VIAC_{15}$[f]	0.34 (< 0.001)	0.15	0.34 (< 0.001)	–0.02	0.33 (< 0.001)	–0.02	0.83 (< 0.001)	0.61 (< 0.001)	0.95 (< 0.001)	0.90 (< 0.001)

[a] $MLCF_{uk}$ = Meat and livestock commission fatness (operating on the UK scale, but expressed on 15-point scale).
[b] $VIAF_{uk}$ = Video image analysis fatness (operating on the UK scale, but expressed on 15-point scale).
[c] $VIAF_{15}$ Video image analysis fatness operating on the 15-point scale.
[d] $MLCC_{uk}$= Meat and livestock commission conformation (operating on the UK scale, but expressed on 15-point scale).
[e] $VIAC_{uk}$ = Video image analysis conformation (operating on the UK scale, but expressed on 15-point scale).
[f] $VIAC_{15}$ = Video image analysis conformation (15-point scale).

Carcase classification needs to be an accurate predictor of SMY% if it is to be an effective mode of carcase evaluation since saleable meat is recognized as the major value component of a carcase. Maximization of SMY% is perhaps more of a target for meat processors than producers at the present time because higher SMY% equates to less waste and greater plant efficiency. SMY% of a carcase will increase when M:B increases at any given FAT% or if FAT% decreases at a constant M:B (Purchas 2003). In order to maximize efficiency, the producer must be able to finish cattle to the target weight, conformation and fat class for the lowest possible cost. Assuming conformation class (carcase shape) relates to the M:B ratio, and fat class is a proxy estimation of total carcase FAT%, carcase classification should be indicative of SMY%, but the correlation between the two end points is variable and often not statistically significant (Keane *et al.* 2000). The poor association between carcase classification and SMY% is problematic both at a farm and at an industry level as it leads to a suboptimal use of resources and a higher level of waste.

The correlation coefficients (r) between the VIA classification and the visual classification firstly adjusted for batch only and secondly for batch, genotype-gender effects and HCW (Tabl3 3.5) indicate that the same characteristic (fatness or conformation) assessed by the two systems were highly correlated. Furthermore, fatness and conformation scores were positively correlated with each other because carcases with higher conformation tend to be fatter. This is mostly due to gender, genotype and weight effects, because after adjusting for genotype-gender, batch and HCW effects, fatness was not correlated with conformation using either the visual or VIA system (Table 3.5). The correlation between $VIAF_{uk}$ and $MLCF_{uk}$ ($r = 0.69$, $P < 0.001$) was stronger than the correlation between $VIAC_{uk}$ and $MLCC_{uk}$ ($r = 0.49$, $P < 0.001$). This suggests that the classifier and VIA are assessing different aspects of carcase shape. The correlations between the two VIA conformation ($VIAC_{uk}$ and $VIAC_{15}$) and fat class ($VIAF_{uk}$, $VIAF_{15}$) traits were high ($r = 0.87$ and 0.92, respectively). The loss in correlation is probably as a result of rounding the 15-point scale into the 8-point conformation and 7-point fatness categories employed for beef carcase classification in the UK.

3.3.3 Prediction of sirloin and fillet weights, yields, and sirloin M:B

The accuracies (R^2% and RSD) and significance of terms used in a range of models for predicting sirloin component weights and SMY% of beef carcases are shown in Table 3.6. As expected, HCW explained the majority of the variation in weight traits (SS and FIL). Batch effects were significant for SS, XSF, BON, SS/HSW (%) and FIL/HSW (%) because differences in carcase processing techniques between days had a large impact on the level of fat trimming (data not shown).

Addition of conformation and fatness predictors in the model offered small improvements in prediction accuracy of SS/HSW (%) and FIL/HSW (%) SMY% traits (models 3 and 4 vs. model 2 in Table 3.6). VIA_{15} (model 6) was able to predict SS/HSW (%) with the highest accuracy overall (R^2 = 58.5%, RSD = 0.35). The difference in accuracy (R^2 values) between VIA and visual carcase classification were small (< 2%). After accounting for genotype-gender, batch and HCW effects, carcase classification offered little in terms of additional accuracy (≤ 5%) for the prediction of FIL/HSW (%). Similarly, the direct prediction of SMY% from VIA (VIA-SMY%) was equally poor at predicting FIL/HSW (%) (Table 3.6).

In terms of SS/HSW (%) prediction, the accuracy of VIA_{15} was marginally higher than MLC_{uk} system. These results are in agreement with R^2 = 57% for VIA and R^2 = 51% for visual classification for predicting yield of cube roll, strip-loin and fillet (very high value cuts) reported by (Drennan *et al.* 2007). The present results are also in agreement with the R^2 = 56% (RSD = 0.30%) for sirloin reported in a recent trial undertaken by the English Beef and Lamb Executive (EBLEX) (personal communication Kim Matthews, EBLEX). Prediction accuracies for M:B obtained from HCW and MLC_{uk} completely explained the genotype-gender effects on M:B ratio (Table 3.6). The accuracies are quite low considering the high value of the sirloin joint relative to the rest of the carcase. The same predictors were also the most accurate at determining the joint composition (percentages of saleable meat, excess fat and bone) of the sirloin region (data not shown).

Table 3.6 Models used to compare manual classification and VIA parameters for predicting loin weight, SMY% and muscle-to-bone ratio on 137 carcases after adjusting for batch effects. The significance of various covariate effects and the main genotype-gender effects are reported.

	Weight			Yield		
[a]Model/Trait	SS (kg)[j]	BON (kg)[k]	XSF (kg)	SS/HSW(%)[m]	FIL/HSW(%)[n]	M:B[o]
Standard Deviation	1.87	0.56	0.40	0.51	0.19	0.46
1. R^2 (%) (RSD)	74.1 (0.71)	47.9 (0.41)	33.0 (0.33)	30.0 (0.43)	5.1 (0.19)	9.6 (0.45)
HCW[a]	< 0.001	< 0.001	< 0.001	0.30	0.60	0.002
2. R^2 (%) (RSD)	81.8 (0.61)	53.7 (0.40)	46.8 (0.30)	53.4 (0.36)	32.4 (0.16)	23.4 (0.42)
HCW[a]	< 0.001	< 0.001	< 0.001	0.23	0.09	0.02
Genotype-gender[b]	< 0.001	0.008	< 0.001	< 0.001	< 0.001	< 0.001
3. R^2 (%) (RSD)	82.5 (0.59)	55.7 (0.38)	51.6 (0.28)	55.0 (0.35)	30.1 (0.16)	24.9 (0.41)
HCW[a]	< 0.001	< 0.001	0.001	0.004	< 0.001	0.66
$MLCC_{uk}$[c]	< 0.001	< 0.001	0.41	< 0.001	< 0.001	< 0.001
$MLCF_{uk}$[d]	0.02	0.17	< 0.001	0.005	0.60	0.14
4. R^2 (%) (RSD)	81.1 (0.61)	51.9 (0.38)	55.4 (0.27)	53.8 (0.36)	22.0 (0.17)	21.7 (0.41)
HCW[a]	< 0.001	< 0.001	< 0.001	0.02	0.01	0.18
$VIAC_{uk}$[e]	< 0.001	0.009	0.80	< 0.001	< 0.001	0.003
$VIAF_{uk}$[f]	0.02	0.09	< 0.001	0.008	0.06	0.06
5. R^2 (%) (RSD)	83.4 (0.58)	57.8 (0.38)	55.6 (0.28)	57.7 (0.35)	36.4 (0.16)	27.7 (0.41)
HCW[a]	< 0.001	< 0.001	0.04	0.68	0.05	0.30
Genotype-gender[b]	0.28	0.28	0.05	0.15	0.03	0.43
$MLCC_{uk}$[c]	0.002	0.002	0.69	0.002	0.03	0.009
$MLCF_{uk}$[d]	0.26	0.27	< 0.001	0.16	0.05	0.70
6. R^2 (%) (RSD)	83.1 (0.60)	55.8 (0.38)	58.4 (0.27)	58.5 (0.35)	36.5 (0.16)	28.4 (0.41)
HCW[a]	< 0.001	< 0.001	0.01	0.45	0.04	0.43
Genotype-gender[b]	0.33	0.30	0.50	0.23	< 0.001	0.33
$VIAC_{15}$[g]	< 0.001	< 0.001	0.37	< 0.001	0.17	0.002
$VIAF_{15}$[h]	0.32	0.52	< 0.001	0.18	0.10	0.88
7. R^2 (%) (RSD)	82.3 (0.60)	53.7 (0.39)	57.1 (0.27)	57.1 (0.35)	35.5 (0.16)	25.1 (0.42)
HCW[a]	< 0.001	< 0.001	0.01	0.85	0.08	0.13
Genotype-gender[b]	0.11	0.47	0.30	0.09	< 0.001	0.35
$VIAC_{uk}$[e]	0.01	0.02	0.33	0.003	0.50	0.05
$VIAF_{uk}$[f]	0.93	0.48	< 0.001	0.81	0.12	0.98
8. R^2 (%) (RSD)	82.7 (0.59)	56.5 (0.37)	48.1 (0.30)	57.7 (0.35)	35.5 (0.16)	28.4 (0.41)
HCW[a]	< 0.001	< 0.001	0.02	0.60	0.02	0.38
Genotype-gender[b]	0.004	0.17	< 0.001	< 0.001	< 0.001	0.09
VIA-SMY%[i]	0.004	< 0.001	0.13	< 0.001	0.08	0.002

Note: all models were corrected for batch (fixed effect, $n = 6$, data not shown).
[a] HCW = Hot carcase weight (covariate effect).
[b] Genotype-gender effect (fixed effect, $n = 6$).
[c] $MLCC_{uk}$= Meat and livestock commission conformation (operating on the UK scale, but expressed on 15-point scale) (covariate effect).
[d] $MLCF_{uk}$ = Meat and livestock commission fatness (operating on the UK scale, but expressed on 15-point scale) (covariate effect).
[e] $VIAC_{uk}$ = Video image analysis conformation (operating on the UK scale, but expressed on 15-point scale) (covariate effect).
[f] $VIAF_{uk}$ = Video image analysis fatness (operating on the UK scale, but expressed on 15-point scale) (covariate effect).
[g] $VIAC_{15}$ = Video image analysis conformation (15-pt scale) (covariate effect).
[h] $VIAF_{15}$ Video image analysis fatness operating on the 15-point scale (covariate effect).
[i] VIA-SMY% = Video image analysis prediction of total carcase saleable meat yield (covariate effect).
[j] SS = Weight of saleable sirloin.
[k] BON = Weight of bone removed from the loin region.
[l] XSF = Weight of excess fat trimmed from the saleable sirloin.
[m] SS/HSW(%) = Yield of saleable sirloin meat (boneless with fat trimmed to 9 mm).
[n] FIL/HSW(%) = Yield of fillet.
[o] M:B = Muscle-to-bone ratio of the sirloin (excluding the fillet).

The accuracies (R^2 values) obtained here for the prediction of sirloin SMY% are low, but it is probable that the prediction accuracies would have been higher if the half carcase SMY% rather than just the sirloin region was available in the current analysis because carcase classification encompasses the whole carcase and is not solely focused on the sirloin region. Several previous experiments that have sought to predict both the whole side and the very-high value cuts have found that classification was considerably

more accurate at predicting the half carcase SMY% than very high value cut yields respectively: R^2 = 68% vs. 51% for the visual and 75% vs. 57% for VIA classification (Drennan *et al.* 2007), R^2 = 70% vs. 29% for visual classification (Drennan *et al.* 2008). Furthermore, the overall accuracy (R^2) of the VBS 2000 across a number of previous experiments was 76% (Section 2.4.7). In the current experiment, carcase classification was not able to measure the SMY% of the sirloin region as accurately as the SMY% of the whole or half carcase reported in other experiments with R^2 values ranging between 60% and 74% (Drennan *et al.* 2007; Drennan *et al.* 2008; Conroy *et al.* 2009; Conroy *et al.* 2010a; Conroy *et al.* 2010b) and even up to 82% (RSD = 1.31%) in a recent trial undertaken by EBLEX (personal communication Kim Matthews, EBLEX).

Total carcase value and the price consumers pay for a package of meat are highly weight-dependent, so there is a case for predicting cut weight. Variation in cut weights is largely explained by HCW, but evaluation based on HCW alone would not account for variations in cattle types (where large differences exist in the proportions of meat, fat and bone). After adjustment for batch effects in the current results, HCW explained 74%, 48% and 33% of the variation in the weights of SS, BON, and XSF in the complete sirloin (CSL) (Table 3.6, components are described in Figure 3.1). This is in agreement with the results of Pabiou *et al.* (2011b) who determined that cold carcase weight could explain 74% (RSD = 2.28 kg) of the variation in very high value cut (rib-roast, strip-loin and fillet) weights. From the current results, it can be seen that HCW does not account for all (or indeed much) of the variation in sirloin XSF or BON weights. Improvements on the weight prediction accuracy were made with the addition of genotype-gender effects (R^2 = 82%) or carcase classification information (R^2 = 83%). Conformation was not a significant predictor for any of the XSF weight models, but EUROP fatness score did account for additional variation over and above genotype-gender effects and HCW (Table 3.6). Carcase classification (fatness score only) did account for an additional 10% of the variation in XSF and BON with VIA_{15} being slightly more accurate than the visual classification (models 6 and 7 vs. model 5, where the R^2 value was 2-3 percentage points higher). VIA-SMY (%) showed similar accuracies for SS R^2 = 83% (RSD = 0.59 kg) and XSF, but accuracies for BON were lower than with carcase classification values.

3.3.4 Supplementing prediction equations with weights of sirloin bone and excess fat

Because FAT% at a constant M:B ratio (and vice versa) determine SMY%, the weights of bone and excess fat removed during the sirloin trimming were tested as additional covariates along with MLC_{uk}, VIA_{uk} and VIA_{15} conformation and fat classes. The purpose was to determine if any additional improvements in SMY% prediction accuracy could be achieved using the extra information. It was found that inclusion of XSF and BON weight as covariates in the current analysis offered very small improvements in prediction accuracy for SS/HSW (%) and FIL/HSW (%) (R^2 increase of 4.5-6.8 percentage points for BON and < 2.5 percentage points for XSF) (data not shown). An early model of whole-side VIA, the BCC-1 (Sørensen *et al.* 1988) had a probe for determining fat depth, yet none of the current whole-side beef VIA-systems have a back fat depth probe, presumably because a measure of fat depth did not increase prediction accuracy. Furthermore, the distribution of fat throughout the carcase varies by genotype, gender, maturity and diet (McPhee *et al.* 2009) making the use of a back fat probe futile unless all (or at least some) of the other factors are taken into account.

3.3.5 Future challenges for beef VIA

A high prediction accuracy for SMY% at a constant muscle-to-bone ratio is dependant on accurate measures of fat percentage (Purchas *et al.* 2002b). Because the VBS 2000 VIA system captures images from the exterior of the half carcase, only subcutaneous fat is visible. Therefore, all predictions of carcase composition are based on the assumption that fat distribution throughout the carcase is consistent, in reality this is not the case, as fat distribution differs between beef and dairy genotypes (Fisher and Bayntun 1984; McPhee *et al.* 2009). Further research is needed to address the differences in fat distribution and how this affects the accuracy with which VIA can predict SMY%.

Most analyses using VIA information from the VBS 2000 system have used predicted EUROP classification variables. Further research is needed to establish whether using raw VIA data (such as primal yield predictions and carcase dimensions) directly, rather than VIA-predicted the EUROP offer improvements in accuracy. Two recent reports have investigated the relationships between various carcase dimensions and yield traits

and presented encouraging findings (Oliver *et al.* 2010; Pabiou *et al.* 2011b), but information in this area is still lacking.

Rather than weighing cuts of beef under abattoir conditions, a non-destructive method of determining carcase composition based on computed tomography (CT) scanning of vacuum packaged primal cuts has been developed (Prieto *et al.* 2009c; Navajas *et al.* 2010a). If CT scanning of primal cuts is performed before any trimming, a more accurate reference measure of carcase composition can be obtained that is unaffected by trimming specifications and transferable between abattoirs. The relationship between carcase composition and SMY% would need to be determined for a range of trimming specifications. A reference system based on CT would also be useful for developing new prediction equations for primal cut yield including some cuts such as the fillet that was poorly predicted using the current EUROP system.

3.4 Summary and conclusions

1. Both VIA and visual classification systems predicted sirloin region weights, yields and M:B with similar accuracies for beef carcases of different genotype and gender, but on balance, the VIA operating on the 15 point scale had slightly higher accuracies.

2. According to previous findings, it is likely that the accuracies obtained in the current experiment would have been higher for both visual and VIA classification systems if the SMY% of the whole or half carcase was available. This is because carcase classification is performed on the whole side of beef, not just the sirloin region.

3. Irrespective of this, both VIA and visual systems were relatively poor at predicting the yield of fillet as there was no statistically significant correlation between fillet yield and carcase classification categories.

4. No substantial improvements in prediction accuracy were gained by including the weight of excess fat trim from the sirloin as a covariate in the current analysis; but the use of sirloin bone weight as an additional covariate did offer promising improvements in prediction of fillet SMY%.

5. Accurate prediction of the carcase sirloin region SMY% is hugely important if the beef industry wishes to adopt a value-based marketing approach to carcase evaluation - especially if meat eating quality parameters are to be included. Further refinement to current carcase evaluation systems is required to address this point.

4 Investigations into relationships between visible-near infrared (NIR) spectra and instrumental meat quality parameters of beef *M. longissimus thoracis*

Presentations based on the results reported in this chapter:

Craigie CR, Ross DW, Maltin C, Purchas RW, Morris ST, Roehe R (2010) The relationship between beef quality and carcass quality attributes measured under commercial conditions. *Proceedings of the British Society of Animal Science annual conference, Belfast* Abstract 129.

Craigie C, Purchas R, Maltin C, Bunger L, Hoskin S, Ross D, Morris S, Roehe R (2010) Video image analysis and near infrared spectroscopy applied to beef carcass evaluation. *Institute of Veterinary, Animal and Biomedical Sciences Research Colloquium, Palmerston North, 25th November 2010*.

Ren J, Marshall S, Craigie C, Maltin C (2012) Quantitative assessment of beef quality with hyperspectral imaging using machine learning techniques. *Proceedings of the 3rd Annual Hyperspectral Imaging Conference, Rome Italy*.

Abstract

Eating quality of meat is known to be a driver for repeat purchases; yet meat of poorer eating quality often reaches consumers because production and processing factors have a considerable impact on meat quality. The ability to identify carcases that are likely to have poorer eating quality would enable processors to improve product consistency and potentially offer a quality guarantee, which could also be included as part of the carcase evaluation process. This experiment aimed to characterize the effects of gender and genotype on instrumental quality of beef *M. longissimus thoracis* (LT) and to evaluate the ability of visible-near infrared (NIR) spectroscopy to predict instrumental measures of beef LT meat quality. Using a sample of LT from 234 cattle of mixed genders and genotypes, it was found that slice shear force values were 31 N higher in bulls than steers ($P < 0.001$), this difference was greater (38.8 N) after adjusting for ultimate pH (pH_{ult}). The colour of steer LT differed significantly to that of bulls and the cooking loss was higher in bulls ($P = 0.03$). The ability of NIR to predict shear force was low, a coefficient of determination (R^2_{pred} = 8%, SE_{pred} = 32.08) was obtained. NIR was more successful at predicting LT lightness (R^2_{pred} = 82%, SE_{pred} = 1.18), redness (R^2_{pred} = 68%, SE_{pred} = 1.55) and yellowness (R^2_{pred} = 48%, SE_{pred} = 1.49), pH_{ult} (R^2_{pred} = 59%, SE_{pred} = 0.15) and the percentage of moisture lost during cooking (R^2_{pred} = 20%, SE_{pred} =

2.75). NIR showed some promise for predicting some instrumental meat quality parameters in beef LT.

4.1 Introduction

The eating quality of meat is an important intrinsic quality cue which partly determines the likelihood of a consumer repeating their initial purchase. Grunert *et al.* (2005) proposed the total food quality model which links consumer expectation (formed at the point of purchase) to the perceived eating quality experience (formed during consumption). According to this model, in a situation where perceived eating quality meets or exceeds expected eating quality, the consumer will be satisfied and is more likely to repeat their purchase. The inherent problem is that the actual eating quality is difficult to infer at the point of purchase. For a number of years, both industry and researchers have long since recognized the need for eating quality information at the point of purchase to aid the consumer purchase decision (Cross and Whittaker 1992).

A number of animal production factors such as growth path (Purchas *et al.* 2002a), genotype (Shackelford *et al.* 1995; Maltin *et al.* 2001; Prieto *et al.* 2011) and gender (Purchas and Aungsupakorn 1993; Sinclair *et al.* 1998) can affect meat quality. Arguably, meat processing techniques have even larger effects on meat quality where a number of interventions such as electrical stimulation (Davey *et al.* 1976; Hwang and Thompson 2001; Hwang *et al.* 2003), chilling rate (Aalhus *et al.* 2001) and aging time (Farouk *et al.* 2009) play an important role in ensuring optimal beef meat quality. Farouk *et al.* (2012) showed how aging disrupts meat structure leading to the improvement of water holding capacity through the "sponge effect". The breakdown of microtubules prohibits water from exiting the meat and there is a corresponding increase in water viscosity due to increased concentrations of peptides from the post mortem proteolysis (Farouk *et al.* 2012). Aging also resulted in an improvement in tenderness due to the breakdown of meat structure (Koohmaraie 1996). Moreover, aging resulted in less variation in shear force values (Prieto *et al.* 2009b; Rosenvold *et al.* 2009) so an increased aging time would lead to greater product consistency.

In an industry full of variation at almost every level of the value chain, variation in eating quality of beef offered to consumers is to be expected. A method of sorting or

grading meat in terms of a predicted eating quality is needed to improve the consistency of beef at the retail level in order to improve customer satisfaction. There are two main drivers for this, firstly, consumers are willing to pay for improved meat eating quality (Lyford *et al.* 2010; Craigie 2011) and, secondly, a growth in demand can be expected through an increase in repeat purchases (Grunert 2005).

The ultimate arbiters of meat eating quality are the consumers, but for reasons of cost and practicality, a number of technological methods are used to assess meat quality, such as slice shear force as a proxy for tenderness (Shackelford *et al.* 1999b), and measures of meat colour, ultimate pH (pH_{ult}) and cooking loss. Slice shear force, and cooking loss require destruction of the sample. Measurements of pH and colour are time consuming and labour intensive. Therefore, these techniques are not ideal for measuring meat quality under abattoir conditions and are unlikely to play a direct role in carcase evaluation.

The slice shear force (SSF) test was developed Shackelford *et al.* (1999b) as an alternative to the Warner-Bratzler shear force protocol (Bratzler 1949) which was too time consuming for a real-time appraisal of meat quality. There has been a limited uptake of other shear force tests such as the MIRINZ tenderometer (Macfarlane and Marer 1966) or the Volodkevich shear force test (Volodkevich 1938). In comparison, to the Warner-Bratzler method, the slice shear method is relatively new, consequently there are very few reports where beef slice shear force has been predicted by NIR spectroscopy (Shackelford *et al.* 2005; Prieto *et al.* 2008; Shackelford *et al.* 2012b). Correlations between slice shear force and Warner-Bratzler shear force in beef *M. longissimus thoracis* (LT) range from $r = 0.66$ ($P < 0.001$) (Lorenzen *et al.* 2010) to $r = 0.80$, $P < 0.001$) (Shackelford *et al.* 1999a) but this correlation may depend on the sampling location within the muscle (Lorenzen *et al.* 2010).

One of the more common factors affecting meat colour is the condition of dark cutting. Meat is described as dry, firm and dark (DFD) when the colour is noticeably darker than normal due to high pH_{ult}. Dark cutting is usually as a result of ante-mortem stress depleting cellular glycogen reserves (Kreikemeier *et al.* 1998). Beef that has a intermediate pH (pH_{ult} between 8.50 and 6.20) is darker in colour and also tends to be tougher due to a curvilinear relationship between pH_{ult} and shear force (Purchas and

Aungsupakorn 1993). Meat that has a high pH_{ult} (> 6.20) is less visually acceptable to consumers than normal coloured meat although differences in terms of tenderness, juiciness and flavour appear minimal (Viljoen *et al.* 2002; Bass *et al.* 2008). The shelf-life of dark cutting meat is significantly reduced compared to normal pH_{ult} meat because there is insufficient acidity to prohibit microbial growth and spoilage (Rousset and Renerre 1991).

Visible-near infrared (NIR) spectroscopy is a safe, non-destructive, fast, and informative technology that has shown some promise for predicting meat quality parameters from a surface scan as reviewed by Prevolnik *et al.* (2004), Prieto *et al.* (2009a) and Weeranantanaphan *et al.* (2011). Certain chemical moieties present in the sample absorb electro-magnetic radiation at certain wavelengths; the NIR spectrometer can detect the presence of chemical compounds based on the absorbance of radiation (Osborne *et al.* 1993). Further explanation of the theory behind NIR is provided in Section 2.7. Many researchers have applied NIR to predict sensory and technological parameters of meat quality with varying degrees of success (Prevolnik *et al.* 2004; Andrés *et al.* 2007; Prieto *et al.* 2009b; Shackelford *et al.* 2012b). A summary of previous research where NIR has been applied to predict instrumental meat quality is provided in Table 2.15, Table 2.16 and Table 2.17.

Furthermore, there have been several attempts to develop NIR prediction equations to predict pH_{ult} in beef LT (Cozzolino and Murray 2002; Andrés *et al.* 2008; Prieto *et al.* 2008; Lomiwes 2008; Rosenvold *et al.* 2009; Yang *et al.* 2010). In terms of instrumental measures of meat quality, the ability of NIR spectroscopy has been variable and researchers have typically not externally validated their calibration equations on new samples, which has been cited as a possible reason for the lack of uptake by the meat industry (Prevolnik *et al.* 2004). NIR is still an emerging technology in the meat industry, particularly in beef, where few reports have applied NIR to whole meat samples under abattoir conditions for the prediction of instrumental eating quality parameters.

The aims of the current experiment were to:

- Characterize the effects of gender and genotype on instrumental measures of beef quality in the *M. longissimus thoracis*.

- Evaluate the effectiveness with which NIR spectroscopy used under abattoir conditions predicted instrumental measures of beef quality in the *M. longissimus thoracis*.

4.2 Materials and methods

4.2.1 Animals

Between March and May 2009, 234 cattle below 30 months of age were selected for inclusion in the experiment at the point of inspection and classification in a commercial abattoir located in central Scotland. Each week during a 10 week period, based on passport breed codes, the following carcases selected for inclusion in the experiment: 4 steers and 4 heifers from the Charolais and Limousin breeds and 4 bulls and 4 steers from the Dairy breeds. After 10 weeks the data set comprised 37 Charolais heifers (CH), 39 Charolais steers (CS), 38 Limousin heifers (LH), 39 Limousin steers (LS), 40 Dairy steers (DS) and 41 Dairy bulls (DB). Abbreviations are also shown with corresponding group in Table 3.1. Differences in numbers in each group were due to a lack of availability of certain genotypes and genders being processed on trial days. Breed codes are entered on the passports by the producer and are derived from the sire breed (Todd *et al.* 2011). All Charolais and Limousin in the current dataset were crossbreds. Of the 82 Dairy animals, 65, 5, 7 and 4 were Holstein-Friesians, Holstein Friesian crosses, Holsteins and British Friesians respectively, according to the breed code descriptions outlined by Todd *et al.* (2011). Cattle were stunned using a captive bolt, exsanguinated, and subjected to electrical stimulation (90 volts for 30 seconds at 10 minutes post mortem) during hide removal. Carcases were dressed to UK specification as described in the beef authentication manual published by Meat and Livestock Commercial Services Limited (www.mlcsl.co.uk). Visual carcase classification for conformation and fatness and was performed by a trained meat and livestock commercial (MLC) services human assessor. Hot carcase weight (HCW) was recorded at the same point as carcase classification.

4.2.2 Carcase cutting and NIR spectra collection

All 234 carcases were quartered between the 10th and 11th ribs at 48 hours post mortem into hind (pistola) and forequarters. A 2.54 cm section of steak containing the LT, associated muscles and subcutaneous fat, was removed from the 11th rib section of each carcase at quartering. Ten replicate NIR spectra (350-1800 nm at 1 nm intervals) were collected using an ASD Qualityspec Pro (ASD Inc., Boulder Colorado) NIR spectrometer fitted with a 63.5 mm active area scanning head by moving and rotating the scanning head on the LT surface as outlined by Prieto *et al.* (2009b). Spectra were collected after allowing the steak to bloom for two minutes (Shackelford *et al.* 2005). The NIR spectrometer was operated using a laptop computer running the Indico Pro program (ASD Inc.). Ultimate pH (pH_{ult}) of LT was recorded 48 hours post mortem at ambient temperature using a temperature compensating Testo 205 pH meter (Testo AG, Lenzkirch, Germany) after calibrating in pH4 and pH7 buffer solutions. The surface of LL exposed at quartering was allowed to bloom for 45 minutes at room temperature before colour measurements (L*, a* and b*) were recorded using a Minolta CR-410, D65 illuminant, 2° standard observer) calibrated against a white tile.

4.2.3 Meat quality assessment

Steaks were placed in sealed plastic bags, transported in cool boxes at 4°C back to SAC Edinburgh and stored overnight at 3°C. The following day (3d post mortem), the LT was trimmed from associated muscles and subcutaneous fat. The LT steaks were weighed and cooked in a clamshell grill (George Forman) to an internal temperature of 71ºC determined with a stainless steel temperature probe (Hanna HI-98509 Checktemp 1) positioned in the geometric centre of the steak. After cooking, the steaks were re-weighed and the cooking loss (CL) was determined by subtracting the cooked weight from the raw weight and expressing the difference as a percentage of the raw weight (n = 166, CL not recorded on weeks 1, 2 or 3). For SSF test, a 50 x 10 mm slice of LT was sheared orthogonal to the muscle fiber axis using a Lloyd TA-plus texture analyser fitted with a flat blunt-end blade as described by Shackelford *et al.* (1999a). The peak slice shear force (SSF) was extracted from the force deformation curve.

4.2.4 Statistical analysis

Descriptive statistics (Table 4.1) were produced using the MEANS procedure of SAS (SAS Inst. Inc., Cary, NC). For each instrumental meat quality trait, least-squares (L-S) means were generated for each genotype-gender group (CH, CS, LH, LS, DS and DB) using a general liner model (GLM) in SAS after adjusting for slaughter day effects as a fixed effect (n = 10) (results not shown). The HCW was fitted as a covariate for all traits and SSF was log transformed ($\log_n$) prior to analysis to ensure a normal distribution. L-S means for SSF were back-transformed to the original scale for ease of interpretation. In order to determine the effect of pH_{ult} on meat quality traits, a second version of the models including pH_{ult} as both a linear and quadratic covariate were fitted. L-S means for the pH_{ult}-adjusted meat quality traits are shown in Table 4.2 for comparison. Three non-orthogonal contrasts were employed to make comparisons between genotypes and genders using "estimate" statements in SAS. The first comparison estimated differences between beef and dairy genotypes within steers, the second comparison estimated differences between steers and young bulls within the dairy genotype and the third comparison estimated differences between steers and heifers within the beef genotypes (Charolais cross and Limousin cross). Highly skewed pH_{ult} data are statistically difficult to analyse because transformations such as a log transformation are insufficient to achieve a normal distribution (Navajas *et al.* 2002). Due to the fact that pH_{ult} data were highly skewed in the current dataset, the genotype-gender effect could not be determined with the same reliability as other meat quality traits, a box-plot (Tukey 1977) was generated in SAS to show the distribution of pH_{ult} data by gender-genotype group (Figure 4.5).

4.2.5 Pre-processing of NIR spectra

Spectra were recorded as absorbance log (1/Reflectance). Plotting all spectra revealed that regions at the extremes of the range (350-1800 nm) contained excessive noise (Figure 4.1). Removing these sections resulted in 495-1600 nm as the working spectra (Figure 4.2).

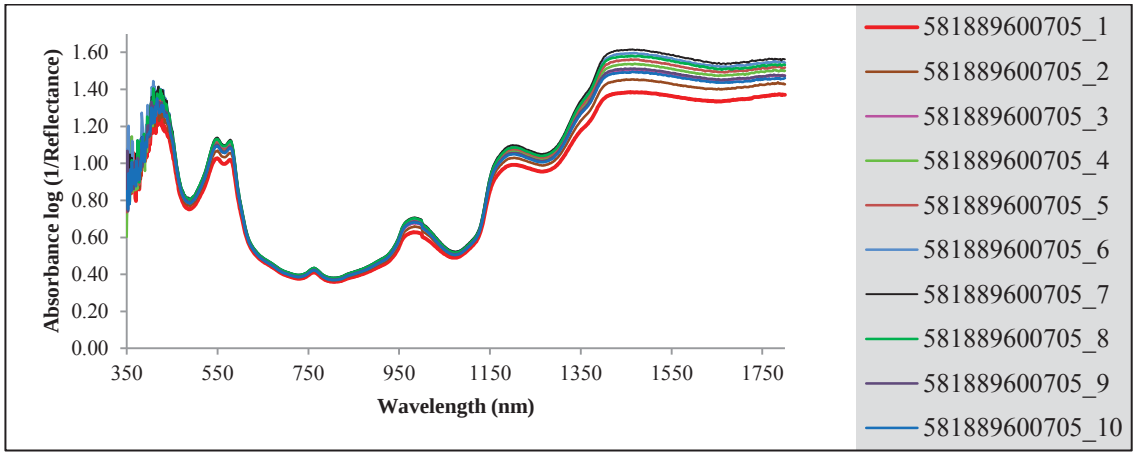

Figure 4.1 A plot of 10 replicate spectra (350-1800 nm) collected from the *M. longissimus thoracis* of one animal showing the noise at the extremes of the spectra.

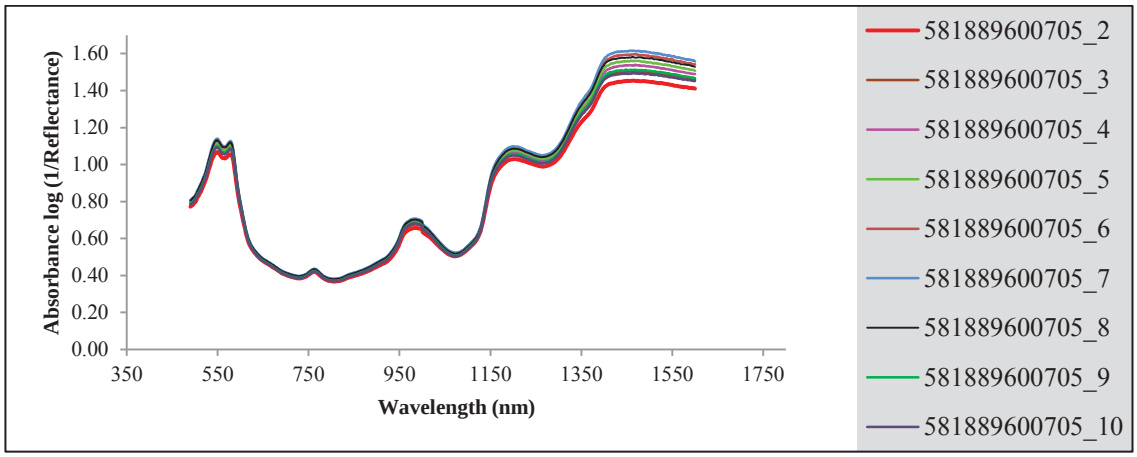

Figure 4.2 A plot of 10 replicate spectra collected from the *M. longissimus thoracis* of one animal after removal of the extreme spectral regions resulting in working spectra of 495-1690 nm.

The ten replicate working spectra for each sample were subject to an initial principal component analysis to detect outliers. A Hotelling T^2 ellipse with the critical test value of $\alpha = 0.25$ was fitted to the principal components scores plot between PC 1 and PC 2 using the Unscrambler (version 10.1) multivariate analysis software (Camo Software AS. Oslo, Norway) (Figure 4.3). Replicates lying outside the Hotelling T^2 ellipse were deemed to be outliers (Krizsan *et al.* 2007). The outlying spectra can be seen in Figure 4.3 and Figure 4.2 as the heavier red line. This approach was taken to simulate a method of determining whether a poor scan could be detected under on-line conditions. Further details of the Hotelling T^2 test are given in Section 4.2.7.

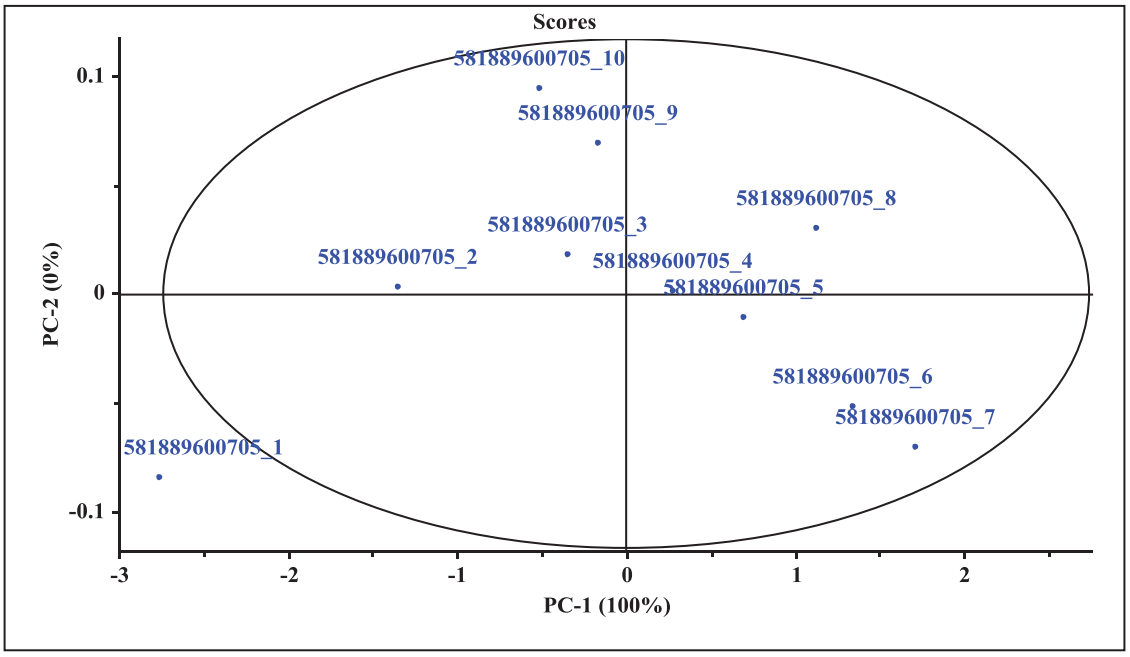

Figure 4.3 A Hotelling T^2 ellipse (α = 0.25) superimposed over the 10 replicates for sample animal ID 581889600705. The first replicate was outside the ellipse, the median spectra for the remaining 9 replicates formed the final spectra for each animal.

On average, approximately 1 out of 10 replicate spectra were sufficiently different from the remaining 9 to be deemed outliers. After removal of outliers, the median value for the remaining replicate spectra was calculated to form the final spectral reading for each meat sample. Scatter effects resulting from interactions between light and structural properties of the sample (such as particles or droplets) (Osborne *et al.* 1993) were visualized by plotting each individual spectra against the average of all spectra (Geladi *et al.* 1985) (Figure 4.4).

Additive effects are seen as different y-axis offsets for different spectra while multiplicative scatter effects are seen as peak intensity dependant spread between different spectra (Esbensen *et al.* 2009). Some scatter effects were present in the spectra, in order to resolve the scatter effects, several spectral pre-treatments were applied including; standard normal variate (SNV), multiplicative scatter correction (MSC) and a second derivative (Table 4.3). Details of the methods are given in Esbensen *et al.* (2009).

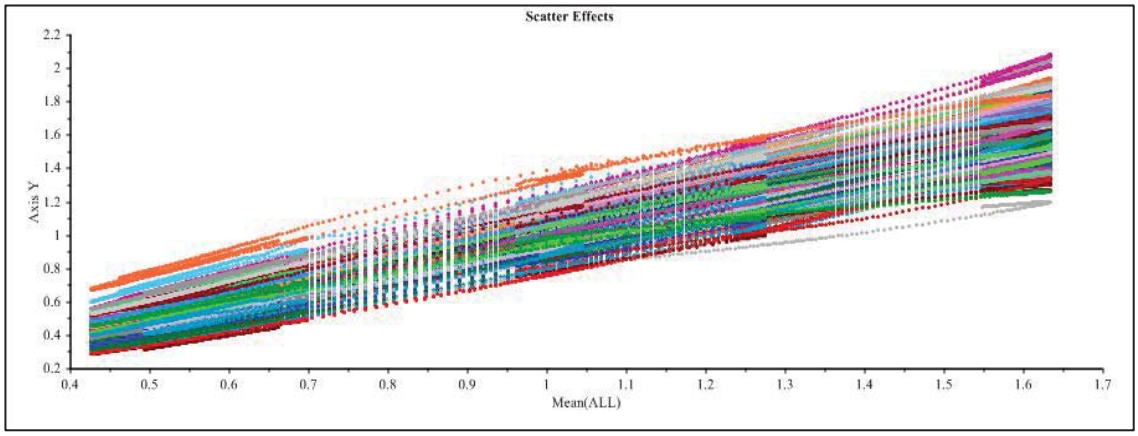

Figure 4.4 A plot showing all individual spectra in a calibration dataset plotted against the average of all spectra in the calibration dataset. The stacking effect is indicative of additive scatter effects and the slight fanning effect is indicative of multiplicative scatter effects.

Plotting each spectra against the average of all spectra (Geladi *et al.* 1985) revealed the SNV and MSC and second derivative transformations had removed the scatter effects and improved the signal to noise ratio for pH_{ult}, SSF and CL, but no pre-treatments were deemed necessary for colour parameters.

4.2.6 Analysis of NIR data

The samples were sorted in ascending order separately for each parameter and every fourth sample was assigned to the prediction dataset, with the intervening three samples being allocated to the calibration dataset as recommended by Williams (2001). The prediction dataset is only used for testing the model as recommended by Naes *et al.* (2002). The calibration model was then subject to full leave-one-out cross-validation where each samples is removed, predicted, and replaced in a sequential manner (Naes *et al.* 2002). Partial least squares regression type 1 was used for predicting instrumental measures of meat quality traits using NIR spectra (495-1600 nm) as explanatory variables.

4.2.7 Detection of outliers

Outliers result in poor model performance and can be attributed either to the reference meat quality measure or to anomalous spectra (Naes *et al.* 2002). Westerhaus *et al.* (2004) describe a strategy for handling outliers. Following this strategy, outliers for the reference meat quality parameter were identified when the calibration, cross-validation

or prediction performance was poor, but samples were only removed if there was a known error with the sample value or where measurements were > 3 SD from the mean of the dataset. Potential outlier spectra were first identified in the same way, through poor calibration, cross validation or prediction performances. The Hotelling T^2 statistic is a generalization of the Student's t-test for multivariate analysis (Hotelling 1931). Further investigation of samples was performed using the Hotelling T^2 ellipse superimposed over a principal component plot (or as a threshold value on a line plot in the prediction dataset) in order to identify samples with high leverage resulting from anomalous spectra. The Hotelling T^2 statistic has a linear relationship to the leverage for a given sample, if upon further investigation, the spectra of the sample was significantly different ($P < 0.01$) from the mean spectra of the sample population based on the F-test (i.e. if a sample falls outside the Hotelling T^2 ellipse ($\alpha = 0.01$)) for any pair of principal components used in the model), the sample was removed. This method is equivalent to the Mahalanobis distance approach used to identify anomalous spectra by Prieto *et al.* (2009b). On the calibration dataset, this step was only undertaken once, as a step-wise approach would also influence the HotellingT^2 ellipse due to changes in the average spectra of the population as outliers are removed.

4.3 Results and discussion

4.3.1 Descriptive statistics

Descriptive statistics for hot carcase weight and meat quality traits are shown in Table 4.1. The mean hot carcase weight (332.6 kg) is similar to the average carcase weight of steers, heifers and young bulls slaughtered in the UK in 2011 which was 342.6 kg (Anon 2012a). Due to highly skewed data the genotype-gender effects on pH_{ult} could not be validly analysed in the same way as other parameters because the normal distribution assumption could not be met. To give an indication of the genotype-gender effects, box-plot of the pH_{ult} data is provided for each group (Figure 4.5). It can be seen that there was a higher incidence of high pH_{ult} in the dairy bull group, and the three steer groups also contained multiple high pH_{ult} samples which is similar to previous reports (Brown *et al.* 1990).

Table 4.1 Descriptive statistics including hot carcase weight, and instrumental measures of meat quality for samples used to assess the effects of sex and genotype on meat quality of beef *M. longissimus thoracis*.

Trait	*n*	Mean	SD	CV (%)	Range
Hot carcase weight (HCW) (kg)	234	332.64	56.01	16.84	185.50-550.00
Slice shear force (SSF) (N)	234	123.43	42.95	34.80	69.83-329.39
Lightness (L*)	234	37.36	2.60	6.96	27.65-45.39
Redness (a*)	234	24.53	2.47	10.07	14.88-32.05
Yellowness (b*)	234	9.04	1.98	21.93	2.74-13.99
Ultimate pH (pH_{ult})	234	5.52	0.23	4.13	5.18-6.74
Cooking loss (CL) (%)	166	18.66	2.89	15.49	9.63-27.47

4.3.2 Gender-genotype effects on meat quality

4.3.2.1 Beef vs. dairy steers

The first contrast compared beef and dairy genotypes within steers (Table 4.2). The HCW (± the standard error) of beef steers was 59.14 ± 8.61 kg greater than the HCW of dairy steers. The cooking loss was 1.88 ± 0.67% greater in beef steers (P = 0.006) and was still present after adjustment for pH_{ult}. Lightness in the LT from DS tended to be 0.86 ± 0.46 units greater than in the LT from beef steers after adjustment for pH_{ult} (P = 0.06). There were no further differences between beef and dairy steers in terms of other instrumental meat quality.

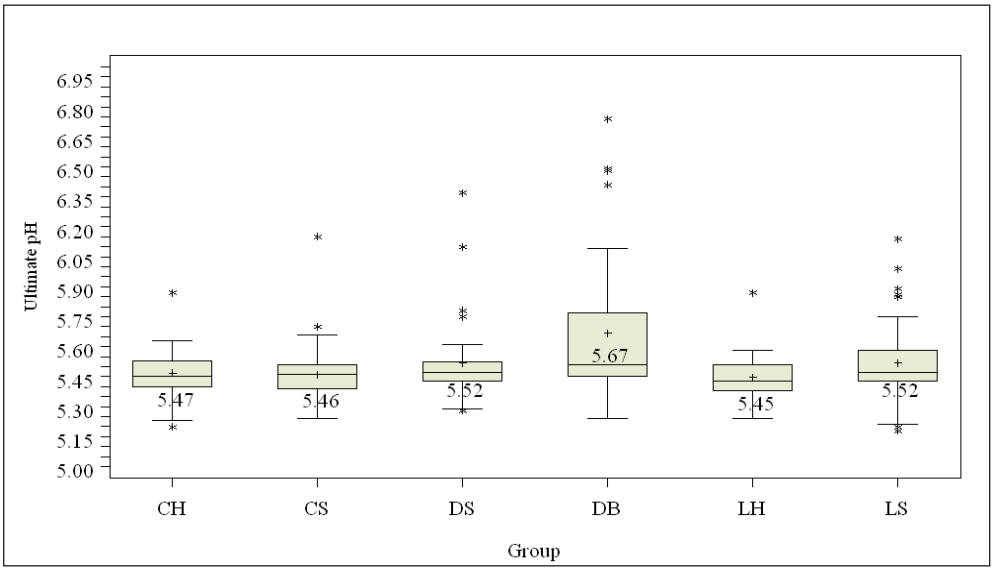

Figure 4.5 Box and Whisker plot of pH_{ult} in the *M. longissimus lumborum* between genotype-gender groups. The length of the box represents the inter-quartile range (IQR), the + sign in the box represents the group mean, the actual mean value is also shown, the horizontal line in the box represents the group median, whiskers represent the maximum and minimum values within the 1.5 x the IQR from the 25th and 75th percentiles * = observations greater than 1.5 x IQR from the 25th or 75th percentile.

4.3.2.2 Dairy steers vs. dairy bulls

The second contrast in Table 4.2 compared steers and bulls within the dairy genotype; there was no statistically significant difference between the bulls and steers for HCW. SSF values were 31 N higher in LT from bulls ($P < 0.001$), the LT of steers was significantly lighter (1.88 ± 0.53 units, $P < 0.001$), redder (1.39 ± 0.46 units, $P = 0.003$) and more yellow (1.29 ± 0.37 units, $P < 0.001$) than LT from bulls. Furthermore the cooking loss was 1.51 ± 0.68% higher in LT from bulls ($P = 0.03$). Ultimate pH was significantly higher in LT from bulls (0.16 ± 0.05 pH units, $P < 0.001$), so this value should be interpret with caution because the data are highly skewed (Figure 4.5). After adjustment for pH_{ult}, the difference in SSF between bulls and steers was greater with LT shear force of bulls being on average 38.8 N higher than steers ($P < 0.001$). The superior tenderness of steers relative to bulls has been reported previously (Reagan *et al.* 1971; Purchas and Aungsupakorn 1993; Purchas and Grant 1995; Purchas *et al.* 2002a). After adjustment for maturity, castration affects the beef muscle fibre composition, enzyme activity and lipid concentration (Schreurs *et al.* 2008a). Differences in some or all of these parameters may offer some explanation for the differences in SSF, meat colour, and cooking loss observed between bulls and steers in the current results.

Unfortunately it was not possible to determine the effect of aging (i.e. maturation of meat) on this difference in the present study, but extended aging is likely to reduce this difference (Sinclair *et al.* 1998). The pH-adjusted model accounted for more variation in SSF and reduced the RSD, and the difference in SSF between the bulls and steers increased indicating that pH_{ult} has a masking effect on the gender effect on SSF. The five highest pH_{ult} observations overall ($pH_{ult} \geq 6.20$) were in the DB group and there was a weak curvilinear relationship observed between pH_{ult} and SSF in the bulls only: $SSF = –91.318 (pH_{ult}^2) + 1032.9 (pH_{ult}) – 2755.4)$, $R^2 = 17.35$, RSD = 51.40. Within the DB group, LT shear force peaked around $pH_{ult} = 5.75$, with SSF values decreasing as pH_{ult} increased beyond that point (Figure 4.6).

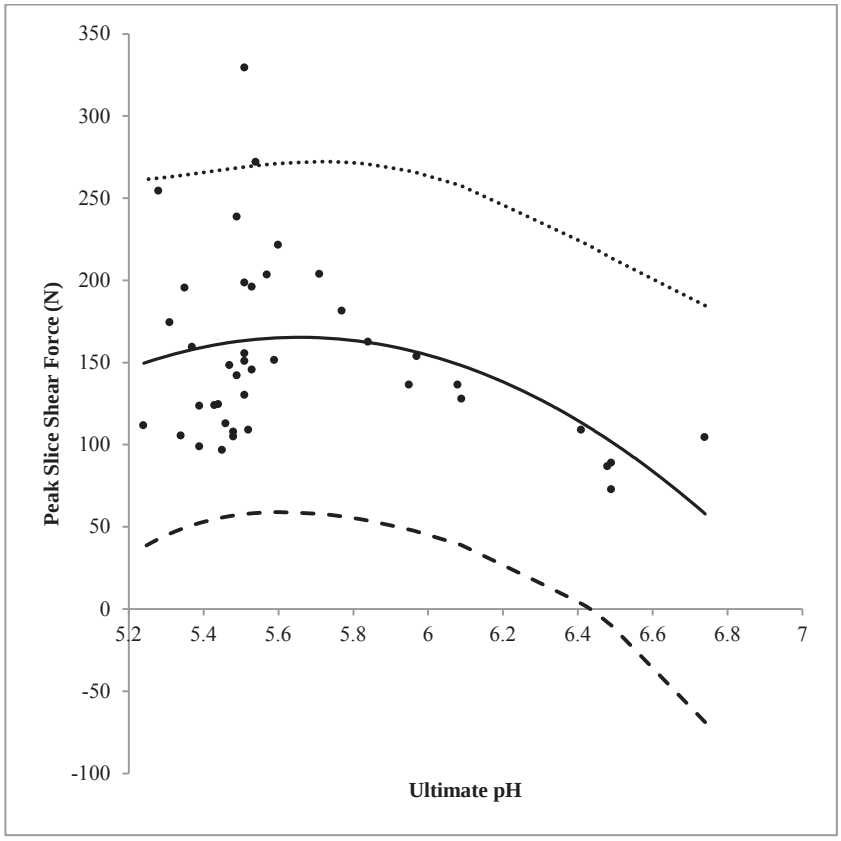

Figure 4.6 The estimated quadratic regression curve (solid line) for the relationship between ultimate pH and the slice shear force values of the *M. longissimus lumborum* in dairy bulls, together with the 95% confidence intervals (dotted and dashed lines). The equation for the relationship is presented in the text.

The fact that SSF actually decreases above about 5.75 pH units, suggests that failing to adjust for pH_{ult} effects could mask the genotype-gender effect on tenderness, particularly when bulls are in the analysis. A curvilinear relationship between shear force and pH_{ult} has been reported in beef (Purchas and Aungsupakorn 1993), lamb (Johnson *et al.* 2005) and venison (Stevenson-Barry *et al.* 1999a). After adjusting for pH_{ult}, the difference in redness and yellowness between DS and DB was no longer statistically significant but the difference in cooking loss between bulls and steers was larger, with LT from bulls having a 2.05 ± 0.64% greater cooking loss than steers (P = 0.001). This is in agreement with reports where LT from bulls had a greater cooking loss than steers (Purchas and Aungsupakorn 1993; Purchas *et al.* 2002a). Further agreement is also seen in Chapter 5 where the *M. longissimus lumborum* (LL) from ram lambs had a greater cooking loss than ewe lambs (Table 5.1) and in Chapter 7 where LL from stags had a greater cooking loss than hinds (Table 7.3).

4.3.2.3 Beef steers vs. beef heifers

The third contrast in Table 4.2, which compared steers and heifers within the beef genotypes (Charolais cross and Limousin cross), showed that the HCW of beef steers was 67.66 ± 7.22 kg heavier than the HCW of beef heifers ($P < 0.001$) (Table 4.2). There were no statistically significant differences in meat quality traits between steers and heifers in the Charolais and Limousin cross genotypes either before or after adjustment for pH_{ult}.

Table 4.2 Least-squares means and pooled standard errors of instrumental measures of meat quality of beef *M. longissimus thoracis* estimated before and after adjustment for pH_{ult} included as both a linear and quadratic covariate. The significance of the three contrasts made between genotype-gender groups is also shown along with the significance of the group effect and hot carcase weight.

Trait[a]	*n*	CH	CS	DS	DB	LH	LS	SE[c]	Contrast[d] A. beef steers vs. dairy steers	Contrast[d] B. dairy steers vs. dairy bulls	Contrast[d] C. beef steers vs. beef heifers	Effects[e] HCW	Effects[e] Gr	Effects[e] pH_{ult}, pH_{ult}^2	R^2 (%)[f]	RSD[g]
		n = 37	*n* = 39	*n* = 40	*n* = 41	*n* = 38	*n* = 39									
HCW	234	319.40	398.04	318.47	310.37	300.53	357.17	7.05	< 0.001	0.41	< 0.001	-	< 0.001	-, -	42.0	44.00
SSF	234	118.90	112.60	109.99	141.40	117.12	111.19	1.05	0.77	< 0.001	0.32	0.52	< 0.001	-, -	18.8	0.27
L*	234	38.26	38.37	38.06	36.18	37.03	36.08	0.40	0.10	< 0.001	0.36	0.06	< 0.001	-, -	23.4	2.35
a*	234	25.46	25.34	24.28	22.89	25.07	24.17	0.35	0.28	0.003	0.20	< 0.001	< 0.001	-, -	36.1	2.04
b*	234	9.95	9.76	9.07	7.78	9.29	8.33	0.28	0.94	< 0.001	0.07	< 0.001	< 0.001	-, -	34.9	1.65
CL	166	20.0	19.0	16.8	18.3	19.2	18.4	0.52	0.006	0.03	0.12	0.69	< 0.001	-, -	23.5	0.03
pH_{ult}	234	5.47	5.49	5.52	5.67	5.45	5.53	0.03	0.91	< 0.001	0.19	0.23	< 0.001	-, -	26.0	0.20
Adjusted for pH_{ult}																
SSF	234	117.76	111.68	108.28	148.14	116.80	109.61	1.05	0.70	< 0.001	0.28	0.57	< 0.001	< 0.001, < 0.001	28.9	0.26[h]
L*	234	38.00	38.23	38.08	36.98	36.68	36.19	0.36	0.06	0.03	0.76	0.15	< 0.001	0.33, 0.47	38.9	2.11
a*	234	25.22	25.21	24.30	23.62	24.73	24.28	0.31	0.26	0.10	0.52	< 0.001	0.002	0.12, 0.21	51.1	1.79
b*	234	9.72	9.63	9.09	8.48	8.97	8.43	0.23	0.84	0.06	0.25	< 0.001	< 0.001	0.07, 0.14	56.3	1.36
CL	166	19.8	18.8	16.9	18.9	19.0	18.4	0.48	0.006	0.002	0.16	0.86	0.001	0.27, 0.20	35.3	2.43

[a] HCW = Hot carcase weight (kg), SSF = Slice shear force (N), L* = Lightness, a* = Redness, b* = Yellowness, CL = Cooking loss (%).

[b] CH = Charolais cross heifers, CS = Charolais cross steers, DS = Dairy cross steers, DB = Dairy cross young bulls LH = Limousin cross heifers and LS = Limousin cross steers.

[c] pooled standard error of the predicted means

[d] Contrast: A = (LS + CS) vs. DS (beef vs. dairy), B = DS vs. DB (bulls vs. steers), C = LIS + CS vs. LH + CH (steers vs. heifers).

[e] Effects (*P* values) HCW = Hot carcase weight (included as a covariate), Gr = genotype-gender group (*n* = 6, included as a fixed effect) also adjusted for batch effects (*n* = 10 where *n* = 235 or 196 or 6 where *n* = 141, included as a fixed effect). The batch effect is not shown. Ultimate pH (pH_{ult}) and pH_{ult}^2 were included as covariates.

[f] Coefficient of determination.

[g] Residual standard deviation.

[h] RSD value for SSF is on the $\log_n$ scale.

4.3.3 Calibration and prediction datasets for NIR analysis

Descriptive statistics for the calibration and prediction datasets are shown in Table 4.3. Arranging the dataset in ascending order for each trait prior to selecting every fourth sample for the prediction dataset resulted in both datasets having a very similar mean and standard deviation (SD). Table 4.1 contains the descriptive statistics for the two datasets combined.

Table 4.3 Descriptive statistics for calibration and prediction datasets used to assess the ability of NIR spectroscopy to predict instrumental meat quality parameters on beef *M. longissimus thoracis*.

	Calibration				Prediction			
Parameter	*n*	Mean	SD	Range	*n*	Mean	SD	Range
Slice shear force (SSF) (N)	175	123.37	42.46	72.77-329.39	59	123.61	44.75	69.83-312.00
Lightness (L*)	175	37.38	2.54	30.64-45.39	59	37.30	2.78	27.65-44.32
Redness (a*)	175	24.55	2.41	18.06-32.05	59	24.46	2.67	14.88-31.40
Yellowness (b*)	175	9.05	1.96	3.70-13.99	59	9.01	2.06	2.74-13.95
Ultimate pH (pH_{ult})	175	5.52	0.23	5.20-6.74	59	5.51	0.23	5.18-6.49
Cooking loss (CL) (%)	124	18.69	2.83	12.57-27.47	42	18.50	3.07	9.63-25.00

In order to test the ability of NIR spectroscopy to predict meat quality traits, models were developed and fully cross validated on the calibration set and the prediction dataset was used as the test set (Table 4.4). No spectral pre-treatment was deemed necessary for the meat colour traits which was also the case in the analysis of Prieto *et al.* (2009b) who also analysed whole meat colour with NIR using the same ASD spectrometer. Most published analyses report either the R^2 for the calibration (R^2_{cal}) or cross-validation (R^2_{cv}) and the standard error of the cross validation (SE_{cv}) as the final indicator of predictive ability, but very few report the R^2 for prediction (R^2_{pred}) or standard error of prediction (SE_{pred}) so comparison on the basis of actual prediction performance is difficult. Because prediction ability is dependant on the variation in the raw data of the trait to be predicted, the ratio of performance deviation (RPD) which is the standard deviation of the Y variable in the calibration dataset divided by SE_{cv} (RPD_{cv}) or preferably the standard deviation of the Y variable in the prediction dataset divided by the SE_{pred} (RPD_{pred}) (Cozzolino *et al.* 2000; Williams 2001; Prieto *et al.* 2009a; Agelet and Hurburgh 2010). An RPD above 8 indicates the model is excellent and can be used with confidence because the standard error of prediction is less than or equal to $1/8^{th}$ the standard deviation in the prediction dataset. RPD values below 2.3 indicate a very poor model and application is not recommended. If the $SE_{pred/cv}$ is

similar to the SD of the reference data (RPD ≈ 1.00), the instrument is not predicting the reference data. (Williams 2001).

4.3.4 Prediction of meat colour with NIR spectroscopy

The R^2 and RPD_{pred} values for the prediction of colour parameters undertaken on 59 samples in the current analysis (Table 4.4) were similar to the results of Andrés *et al.* (2008) who obtained R^2_{cv} = 75% (SE_{cv} = 1.36, RPD_{cv} = 2.07) for L*, R^2_{cv} = 29% (SE_{cv} = 1.28, RPD_{cv} = 0.90) for redness and R^2_{cv} = 46% (SE_{cv} = 0.99, RPD_{cv} = 1.37) for yellowness for 30 *M. longissimus thoracis* (LT) samples from bulls after allowing the meat to bloom for 60 minutes.

Table 4.4 Performance of NIR calibration equations showing the coefficient of determination (R^2) and standard error (SE) for calibration, cross-validation and prediction phases for predicting instrumental meat quality in beef *M. longissimus thoracis*.

			Calibration			Cross-validation			Prediction			
Trait[a]	Pre-treatment[b]	PC[c]	*n*[d]	R^2 (%)	RMSE[e]	R^2 (%)	SE	RPD[f]	*n*[g]	R^2 (%)	SE	RPD[h]
SSF (N)	MSC	3	175	14.9	39.05	8.8	40.78	1.04	57	8.2	32.08	1.04
L*	none	3	175	72.0	1.34	70.6	1.39	1.84	59	81.9	1.18	2.35
a*	none	5	175	40.5	1.85	34.0	1.96	1.23	59	67.6	1.55	1.72
b*	none	2	175	53.6	1.33	48.4	1.42	1.38	59	47.6	1.49	1.38
pH_{ult}	SNV, 2nd D	7	175	88.4	0.08	59.5	0.14	1.57	58	59.3	0.15	1.54
CL (%)	MSC	7	120	40.2	2.08	19.00	2.46	1.15	42	20.0	2.75	1.12

[a] SSF = Slice shear force, L* = Lightness, a* = Redness, b* = Yellowness, pH_{ult} = Ultimate pH, CL = Cooking loss.
[b] Pre-treatments applied to the spectra prior to PLS regression analysis and prediction, SNV = standard normal variate, 2nd D = second derivative (gap-segment: gap-size = 5, segment size = 1), MSC = multiplicative scatter correction.
[c] PC = number or principal components used in the regression.
[d] *n* = number of samples included in the calibration and cross-validation phases.
[e] RMSE = root mean square error.
[f] RPD = ratio performance deviation is the SD of the Y variable in the calibration dataset (after removal of outliers) divided by the SE_{cv}.
[g] *n* = number of samples included in the prediction phase.
[h] RPD = ratio performance deviation is the SD of the Y variable in the prediction dataset (after removal of outliers) divided by the SE_{pred}.

Prieto *et al.* (2009b) obtained R^2_{cv} = 83% (SE_{cv} = 0.96, RPD_{cv} = 2.47) for L*, R^2_{cv} = 76% (SE_{cv} = 0.95, RPD_{cv} = 2.02) for a* and R^2_{cv} = 69% (SE_{cv} = 0.84, RPD_{cv} = 2.48) for b* for beef LT samples after allowing the meat to bloom 45 minutes. The RPD_{pred} for L* in the current analysis (2.35) was similar to the RPD_{cv} of 2.47 reported by Prieto *et al.* (2009b), but performance for predicting a* and b* were not as strong as the results of Prieto *et al.* (2009b). The fact that NIR spectra are collected almost immediately after exposing the meat surface to air, whereas colour traits are measured after blooming for around 45 minutes is a likely reason for the less than perfect correlation between spectra and the reference measures of L*, a* and b*. An example of the blooming effect can be seen as the difference between exposure times t_0 minutes and t_{60} minutes in the results

of Andrés *et al.* (2008) where the largest changes occurred in redness and yellowness while lightness only marginally increased with blooming time. Moss *et al.* (2010) also showed that blooming time had significant effects on NIR spectra below 1350 nm, particularly in the visible region. The spectra collected after at least one hour blooming had slightly better ability to predict Warner-Bratzler shear force on beef aged for 14 and 21 days (Moss *et al.* 2009). The variation between the current results and those of Prieto *et al.* (2009b) may be due to the fact that data were collected from different processing plants or different criteria for the exclusion of data, although predictive performance varies considerably between experiments where NIR has been used to predict colour parameters on intact meat (Prieto *et al.* 2009a).

4.3.5 Prediction of ultimate pH with NIR spectroscopy

One sample (Sample 155) was removed from the prediction dataset as it was an outlier with high deviation (Figure 4.7). Plotting the spectra for sample 155 against the average for all samples in the pH_{ult} prediction dataset shows that the spectra become increasingly different as the wavelength increases (Figure 4.8).

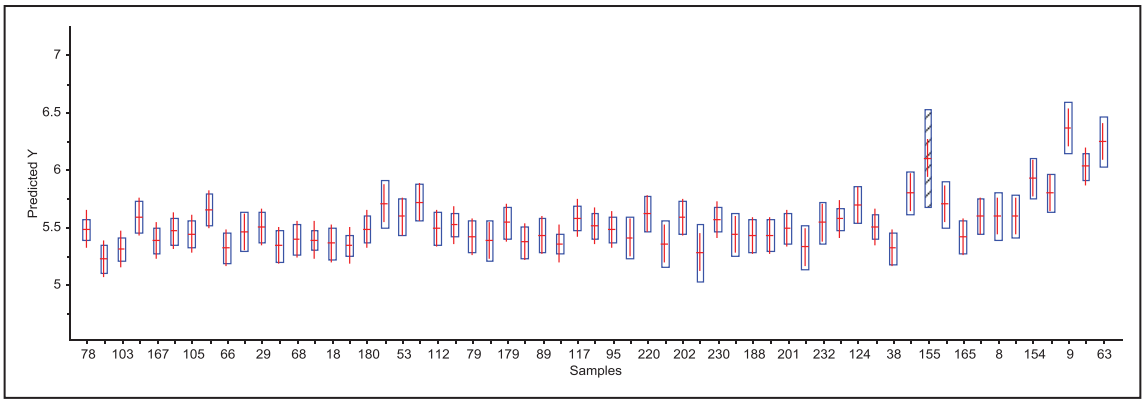

Figure 4.7 The prediction of *M. longissimus thoracis* pH_{ult} showing the predicted value as the horizontal red line, Sample number 155 is marked with diagonal lines. Boxes around the predicted value indicate the deviation which is estimated as a function of the global model error, the sample leverage and the sample residual X variance. A large deviation indicates that the sample is not similar to the samples used to make the calibration model and consequently can be considered a prediction outlier.

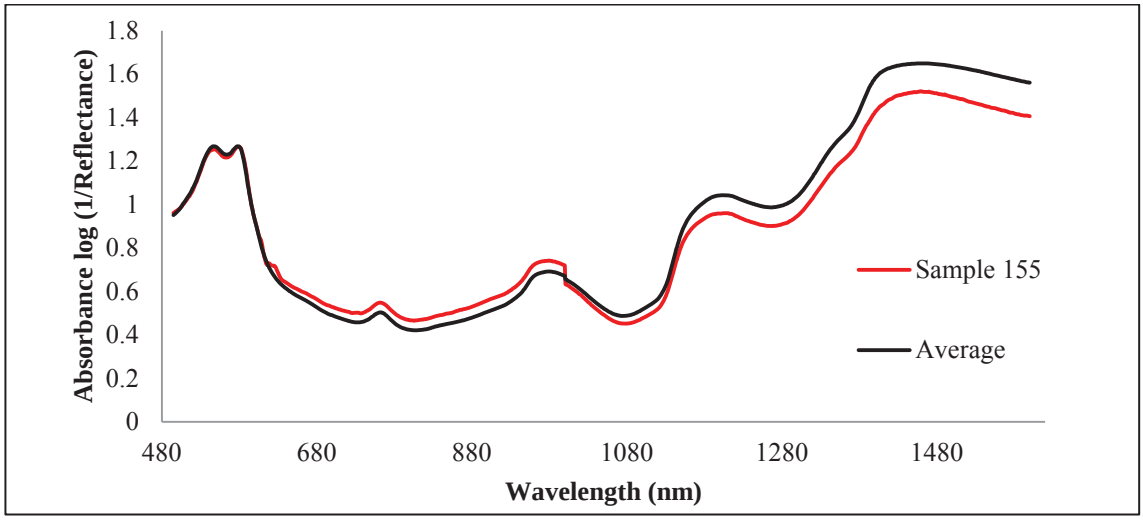

Figure 4.8 A plot of the spectra from Sample 155 along with the average spectra of the 59 samples in the calibration dataset for ultimate pH. The spectra from Sample 155 diverges further from the average spectra as the wavelength increases.

Retaining sample 155 reduced the R^2_{pred} associated with the prediction of pH_{ult} to 50.9% (SE_{pred} = 0.16, RPD_{pred} = 1.38). The R^2_{cal} for pH_{ult} obtained in the present analysis (Table 4.4, Figure 4.9) is lower than the R^2_{cal} of 97% (SE_{cv} = 0.10, RPD_{cv} = 3.17) reported by Andrés *et al.* (2008) on a sample of 30 bulls. The distribution of observations in Figure 4.9 indicates that the young bulls are largely responsible for the correlation obtained in for pH_{ult}. The R^2_{cal} obtained in the current analysis is slightly higher than the R^2_{cal} values of 81% (SE_{cv} = 0.18) obtained on 100 beef samples reported by Cozzolino and Murray (2002) and 85% (SE_{cv} = 0.20) obtained on 26 Hereford steers (Rosenvold *et al.* 2009). Prieto *et al.* (2008) reported an R^2_{cal} of 41%, SE_{cv} = 0.06, RPD_{cv} = 1.12 for pH_{ult} on 53 steers and Lomiwes (2008) reported an R^2_{cv} of 20%, (SE_{cv} = 0.13) for pH_{ult} on 85 cattle, but NIR spectra were collected on pre-rigor beef in the latter experiment. Predictive ability for pH_{ult} in these two reports was much lower than the R^2_{cal} obtained in the current calibration (Table 4.4).

The current analysis contains a much larger number of animals of various breeds and genders than any previous report on NIR to predict pH_{ult} in beef. Rosenvold *et al.* (2009) did split their dataset into calibration and validation datasets but the validation dataset comprised of multiple measurements on 14 Hereford steers. Considering the ability of NIR to segregate samples with high pH_{ult} values which may be dark cutting, NIR was able to correctly identify all five samples in the prediction dataset that had pH_{ult} values > 5.80, a threshold that has previously been useful for identifying toughness

in beef (Jeremiah *et al.* 1991). Furthermore, only one sample with a reference pH_{ult} value below 5.80 was classified as having a pH_{ult} value equal to 5.80 which was therefore misclassified (Figure 4.10).

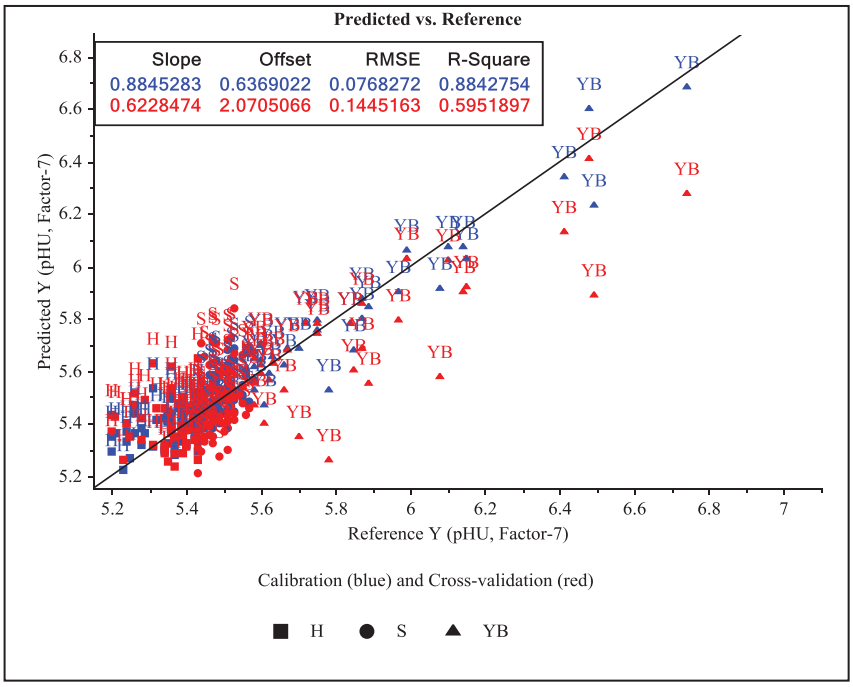

Figure 4.9 Calibration (blue) and cross-validation (red) for *M. longissimus thoracis* pH_{ult} also showing the gender of the samples, H = heifer, S = steer and YB = young bull. The distribution of observations shows that the high pH_{ult} meat is entirely from the young bulls.

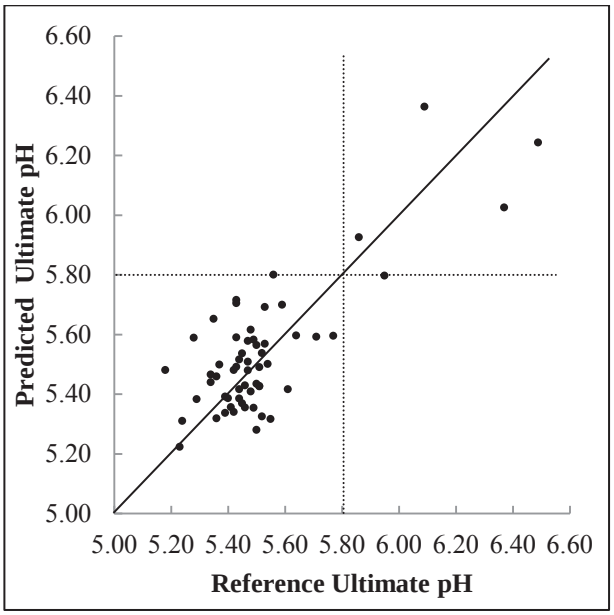

Figure 4.10 Prediction of *M. longissimus thoracis* pH_{ult} from NIR spectra on 59 samples showing that the model could correctly identify the five samples ≥ pH_{ult} = 5.80.

4.3.6 Prediction of cooking loss with NIR spectroscopy

After removing three outlier samples from the calibration dataset, the ability of NIR to predict cooking loss was poor in the current results (R^2_{pred} = 20%, SE_{pred} = 2.75, RPD_{pred} = 1.12) (Table 4.4). All outliers were spectral outliers ($P < 0.01$) and retaining these observations resulted in total failure of the model, but in terms of performance, the RPD_{pred} (1.12) was similar to previous reports (Leroy *et al.* 2004; Prieto *et al.* 2009b). Leroy *et al.* (2004) reported an R^2_{cal} of 25% (SE_{cv} = 2.31%, RPD_{cv} = 1.13) using 101 cow and 88 bull LT samples aged for two days prior to cooking. Andrés *et al.* (2008) reported an R^2_{cal} of 20% (SE_{cv} = 0.08%, RPD_{cv} = 1.01) using LT samples from 30 bulls. Prieto *et al.* (2008) reported an R^2_{cal} of 14% (SE_{cv} = 1.61%, RPD_{cv} = 1.04) based on LT samples aged seven days from 53 steers and R^2_{cal} of 0.001% (SE_{cv} = 2.45%, RPD_{cv} = 0.97) using LT aged for three days from young cattle, although the NIR spectra were collected on homogenised meat samples as opposed to the intact LT muscle, Prieto *et al.* (2009b) reported an R^2_{cv} of 23% (SE_{cv} = 2.35%, RPD_{cv} = 1.14) based on 130 LT samples aged 14d. It is possible that the poor prediction ability of NIR for cooking loss is a result of heterogeneity in the samples, possibly due to fat forming a barrier to cooking loss (Hornstein *et al.* 1960), or the fact that smaller carcases yield smaller

samples of LT at a constant thickness which have a higher surface area to volume ratio resulting in greater cooking loss. This is likely to be of importance when the entire slice of LT is cooked without any standardization of dimensions which was the case in the current analysis.

4.3.7 Prediction of slice shear force with NIR spectroscopy

As was mentioned previously, there are few reports where the ability of NIR to predict SSF of beef has been examined. Two extremely high SSF values were removed from the prediction dataset because they were > 3 SD from the mean. In the first instance, results obtained in the current analysis (R^2_{pred} = 8.2%, SE_{pred} = 32.08) (Table 4.4) appear much poorer than the R^2_{cal} = 54%, SE_{cv} = 46.49) reported previously by Prieto *et al.* (2009b) on 194 Aberdeen Angus - Limousin crossbred steers (*n* = 128) and heifers (*n* = 66), despite using the same NIR spectrometer and methodology as used by Prieto *et al.* (2009b). Although samples came from different abattoirs, the coefficient of variation (34.7%) published by Prieto *et al.* (2009b) was similar to that from the current dataset (34.4%), but the mean SSF value was much lower (123.37 N, SD = 42.46) in the current dataset than the 192.45 N (SD = 69.77) in the experiment of Prieto *et al.* (2009b). Even if the variation in the current prediction dataset is taken into account using the RPD statistic, the prediction performance for shear force was still poorer (RPD_{pred} = 1.04) than reported by Prieto *et al.* (2009b) where the RPD_{cv} values for 3d SSF was 1.25 and 14d SSF was 1.14. This illustrates how the SD affects the RPD value; higher SD values result in higher RPD values. Prieto *et al.* (2009b) excluded 18 samples from their analysis either where the Mahalanobis distance was ≥ 3.0 or where the predicted value in the cross-validation was ≥ 2.5 times the standard error of the estimate. Exclusion based on the latter could lead to an upward bias of the R^2_{cal} and downward bias of the SE_{cv} because removing too many samples with high residuals makes the model look better (Westerhaus *et al.* 2004). Applying the later criteria on the current dataset did not improve the model performance because samples could not be justified as outliers (data not shown). On a prediction dataset of 30 steaks from different animals, Moss *et al.* (2009) reported and R^2_{pred} values of 5.6% (SE_{pred} = 0.23) and 20.7% (SE_{pred} = 0.54) for Warner-Bratzler shear force on 14d and 21d aged beef respectively, but they did not report the standard deviations of the prediction dataset so the RPD_{pred} could not be calculated. Shackelford *et al.* (2005) recorded NIR spectra and slice shear force on 292

carcases from two plants, the dataset was split in half into calibration and validation datasets. The breed and sex information for the carcases was not given so it is difficult to draw a comparison on that basis, but the coefficient of variation for SSF was similar (36.31%) to the current dataset. Shackelford *et al.* (2005) obtained an R^2_{cal} of 38% and R^2_{pred} of 22% for 14d SSF but they did not report the standard errors of prediction so comparison based on the RPD_{pred} is not possible. The model was re-validated in terms of the ability to segregate samples into two SSF tenderness categories above and below the median shear force value (Shackelford *et al.* 2012b). The mean predicted SSF of the two categories is then tested for a significant difference, the "predicted tender" (≤ the median shear force) category has a lower mean tenderness value than the "not predicted tender" group (Shackelford *et al.* 2012a).

In terms of model development, Prieto *et al.* (2008) used partial least squares regression and Shackelford *et al.* (2005) used a form of multiple regression to determine a 10 variable prediction equation. Partial least squares regression was also the method used in the current analysis for the prediction of SSF, but predictive ability was poor which was partly due to the dataset as evidenced by the RPD_{pred} value. Despite the fact that there are few reports using the SSF method as used in the current analysis and in the analysis of Prieto *et al.* (2009b), 12 studies have used NIR to predict Warner-Bratzler shear force (the details of these is given in Table 2.15, Table 2.16 and Table 2.17). The average RPD_{cv} values reported for these 12 studies for LT Warner-Bratzler shear force is 1.20 (RPD_{cv} ranging from 1.05 to 1.46), the results of Park *et al.* (1998) were excluded because carcases were selected based on Warner-Bratzler shear force values to maximize variation. The average RPD_{cv} is similar to the RPD_{cv} = 1.25 and 1.14 for 3d and 14d SSF reported by Prieto *et al.* (2009b) and higher than the RPD_{pred} = 1.04 obtained for 3d SSF in the current analysis.

NIR was unable to identify the toughest samples in the dataset (data not shown) which was expected given that the R^2_{pred} was so low. There are a number of possible explanations for this, firstly the standard deviation of the reference shear force data was very low compared to previous experiments, and thus there was little variation to predict. Another possible explanation was the skewed distribution of shear force values. The reference shear force data had high frequency of samples with low shear force values and a low frequency of samples high shear force values. As a result there were

insufficient samples with high shear force values to build a stable prediction equation, because the cluster of lower shear force values dominated the model.

4.3.8 Future directions for NIR analysis on beef

A possible approach to improving the stability of NIR prediction equations could be to flatten the distribution of reference samples so that calibration could be performed on an even distribution of samples across the range of shear force variables (Williams 2001). For this to be possible, a larger number of NIR scans and reference shear force values would be needed, validation should be performed externally on a dataset with the typical skewed distribution encountered in a commercial environment. Some very recent analysis on the current dataset using a novel machine learning approach with principal components followed by support vector machine regression has shown more promise (Ren *et al.* 2012). They described the method and reported a correlation of 0.53 (validated by cross-validation) between slice shear force and NIR spectra (Ren *et al.* 2012). More work is needed to determine how the method performs on a prediction dataset.

4.4 Summary and conclusions

1. The pH_{ult} data were highly skewed and transformations were ineffective for obtaining a normal distribution thus the results from the analysis of pH_{ult} data using a general linear model need to be interpreted with care. These results indicated that LT from bulls had significantly higher pH_{ult} values than steers.

2. Cooking loss was significantly greater in beef steers than dairy crossbred steers.

3. Despite having similar carcase weights, the LT from bulls had a higher shear force, and higher cooking loss than LT from steers. The LT from bulls was also darker, less red and less yellow than that from steers.

4. The relationship between pH_{ult} and LT shear force for bull samples was found to be curvilinear with shear force decreasing above 5.75 pH units.

5. Failing to account for pH_{ult} effects could mask genotype-gender effects for tenderness, colour and cooking loss.

6. There were no statistically significant differences in the analysis of meat quality between beef steers and beef heifers.

7. The sample size of the current analysis is considerably larger and more heterogeneous than the datasets of most previous reports where NIR has been applied to predict meat quality parameters.

8. NIR spectroscopy could predict L* (R^2_{pred} of 82%, RPD_{pred} = 2.35), a* (R^2_{pred} of 68%, RPD_{pred} = 1.72), and b* (R^2_{pred} of 48%, RPD_{pred} = 1.38).

9. The model for predicting pH_{ult} with NIR spectroscopy was much stronger in the calibration (R^2_{cal} = 88%) than in the cross-validation and prediction phases (R^2_{pred} = 59%). The model was able to correctly identify the 5 samples in the prediction dataset with pH_{ult} values higher than normal (≥ 5.80), although one normal pH sample was misclassified as having a high pH_{ult}.

10. The ability of NIR to predict slice shear force was low compared to values reported in previous analyses, this is partly due to the low SD of the reference data.

11. The ability of NIR to predict cooking loss was similar to previous reports.

12. An alternative method of data analysis for slice shear force based on support vector machine regression has shown promising improvements over the partial least squares regression approach which was used in the present study.

5 Effect of sex and TM-QTL genotype on some carcase and meat quality traits in Texel ewe and ram lambs

Presentations and publications based on the results reported in this chapter:

Craigie CR, Lambe NR, Richardson RI, Haresign W, Maltin CA, Rehfeldt C, Roehe R, Morris ST, Bunger L (2012) The effect of sex on some carcass and meat quality traits in Texel ewe and ram lambs. *Animal Production Science* **52**, 601-607.

Craigie CR, Lambe NR, Macfarlane JM, Maltin C, Morris ST, Roehe R, Bunger L (2011) Effect of the Texel muscling quantitative trait locus (TM-QTL) and sex on meat quality parameters of the *semimembranosus* muscle of purebred Texel lambs. *Proceedings of the British Society of Animal Science annual conference, Nottingham, UK*. Abstract 067.

Abstract

Much of the past research into gender effects on lamb meat quality has focused on comparing ram lambs with castrated males, but more recent comparisons between ram and ewe lambs have yielded variable results. The first objective of the current research was to compare instrumental meat quality parameters of *M. longissimus lumborum* (LL), and *M. semimembranosus* (SM) from pasture-fed Texel ram (n = 94) and ewe (n = 114) lambs slaughtered at an average age of 144 days in a commercial abattoir. The second objective was to characterize the effects of the Texel-Muscling quantitative trait locus (TM-QTL) on SM quality on a subset of lambs (n = 143) with known TM-QTL genotypes. After aging carcases for between seven and nine days, LL and SM were significantly tougher (higher shear force values) for ram compared to ewe lambs (P < 0.001). LL from rams had significantly lower intramuscular fat percentage (IMF%), and higher moisture content (Moist%) than LL from ewes. Differences in LL IMF%, Moist% or ultimate pH (pH_{ult}) did not explain the sex effect on LL shear force when tested individually or together as additional covariates in the model. Ram SM was lighter in colour (higher L*) and had a higher cooking loss than that of ewes (P < 0.001). There was no evidence of a TM-QTL genotype effect on instrumental meat quality parameters of SM and no evidence of any sex by genotype interactions. The correlations between some of the traits within and between muscles clearly differed between the sexes. Finishing ram lambs to the specifications used in this experiment

resulted in meat with relatively minor, but statistically significant differences in quality relative to that from ewe lambs.

5.1 Introduction

Research has shown that tenderness is highly correlated to overall liking in cooked lamb meat (Thompson *et al.* 2005). The supply of consistently tender lamb should meet consumer expectations and result in repeated purchases of lamb meat (Grunert 2005). Given that lamb gender can affect retail acceptability (Jeremiah *et al.* 1993) and meat eating quality (Dransfield *et al.* 1990), extensive research has investigated the differences in the quality of meat from ewe lambs and castrated male lambs, but there is considerably less research focusing on the differences between entire ram lambs and ewe lambs in terms of meat quality. Ram lambs have a higher growth rate, heavier carcases at a set age and are less fat than ewe lambs (Dransfield *et al.* 1990; Lee *et al.* 1990). Nonetheless, many producers castrate male lambs to reduce unwanted pregnancies, aggressive behaviour, and to improve marketability (Stafford and Mellor 2010). The resulting castrates are intermediary to ewes and rams in terms of production efficiency and eating quality (Dransfield *et al.* 1990; Okeudo and Moss 2008). Despite this, the financial gains achieved by castrating ram lambs are highly variable (Fisher *et al.* 2010) and there are concerns for animal welfare with castration and especially with some castration methods (Stafford and Mellor 2010).

Recent research into the sex effects on lamb meat quality, Lambe *et al*. (2010b) found no significant differences in *M. longissimus lumborum* (LL) or *M. vastus lateralis* shear force between pasture-fed ram and ewe lambs for meat aged for seven days. Navajas *et al*. (2008) reported that there were no differences between ram and ewe LL and *M. semimembranosus* (SM) in sensory toughness, although consumers preferred ewe LL to ram LL, there was no difference between sexes for overall liking of SM. In contrast, Johnson *et al*. (2005) found LL and SM shear force values were higher in Texel-cross ram lambs than ewe lambs raised and slaughtered under the same conditions, but the differences were relatively small. Lind *et al.* (2011) found that lamb sex affected a variety of LL sensory parameters (including tenderness) in five month old Norwegian White sheep with meat from ram lambs being significantly tougher than ewe lambs

when finished on rye-grass pasture for 24 days pre-slaughter, but not after 44 days grazing on rye-grass pasture (Lind *et al.* 2011).

A single copy of the Texel muscling quantitative trait locus (TM-QTL) located on ovine chromosome 18 increases loin muscle depths (assessed by ultrasound) by 1.2-2.0 mm (mean depth of 28.7 mm) which equates to a 4-8% increase in loin muscle area (Walling *et al.* 2004; Lambe *et al.* 2010b). Macfarlane *et al.* (2009) reported that a single copy of the TM-QTL resulted in a 4% increase in loin muscle depth at the third lumbar position which resulted in a 7% increase in the loin muscle weight of crossbred lambs, with no apparent effects on other cuts or the saleable meat yield of the carcase. Macfarlane *et al.* (2010) reported that carriers of TM-QTL in pure-bred Texel lambs had between 4 and 11% greater *M. longissimus* muscle areas and that the inheritance mode of the allele was polar over-dominant meaning that the effect of the TM-QTL was apparent only when a single copy was inherited from the sire. Macfarlane *et al.* (2012) also reported that lambs carrying two copies of the TM-QTL had between 7 and 15% greater live weights over a range of ages compared to non-carriers and that the carcases of lambs carrying two copies of TM-QTL were 9% higher than the non-carriers. Overall, the loin weights are between 4 and 14% higher in lambs carrying at least one copy of the TM-QTL in purebred and crossbred Texel lambs (Walling *et al.* 2004; Macfarlane *et al.* 2009; Macfarlane *et al.* 2010) but other muscles seem to be unaffected (Macfarlane *et al.* 2009).

A more extreme form of muscular hypertrophy observed in lambs carrying the Callipyge mutation has been associated with increased shear force in LL, and to a lesser extent in the SM (Duckett *et al.* 1998; 2000). In terms of the effects of TM-QTL on meat quality, Lambe *et al*. (2010b) reported a significant sex-by-genotype interaction where LL from crossbred rams with one copy of the TM-QTL allele had significantly higher shear force values than other genotypes, but the effect disappeared after 7d aging (Lambe *et al.* 2010a). Furthermore, there were no such effects on *M. vastus lateralis* in that study. Lambe *et al*. (2011) reported that TM-QTL genotype had no significant effects on instrumental meat quality parameters of LL and *M. vastus lateralis* of purebred Texel lambs. The contrasting effects of TM-QTL genotype on LL shear force between the cross-bred Texels and the pure-bred Texels may possibly be due to any number of factors, as the experiments were in different years. An epistatic interaction

with the genetic background of the lambs used in the respective experiments is another possible explanation. An example of an epistatic interaction between a muscling genotype and genetic background can be seen when comparing South Devon and Belgian Blue cattle, both breeds share the same mutation in the myostatin gene, but only the Belgian Blue cattle show a double-muscled phenotype (Wiener *et al.* 2002). The effect of the TM-QTL genotype on instrumental meat quality parameters of SM has not been investigated, so before the TM-QTL can be recommended to be used in the industry as a means of increasing LL yield, effects of TM-QTL genotype on SM meat quality should be investigated.

The aims of the current experiment were to:

- Evaluate sex effects on lamb meat quality parameters of *M. longissimus lumborum* and *M. semimembranosus* in Texel ram and ewe lambs.
- Characterize the effect of the TM-QTL on meat quality parameters of *M. semimembranosus*.

5.2 Materials and methods

5.2.1 Animals

In 2009 Texel dams (n = 181) on two farms were mated to seven Texel sires that carried at least one copy of the TM-QTL allele. Three of the sires were used on both farms in accordance with the objectives of a larger experiment investigating the effects of the TM-QTL on lamb performance and eating quality (Macfarlane *et al.* 2010; Lambe *et al.* 2011). Meat samples were available from ewe (n = 114) and entire ram lambs (n = 94) that were finished together on pasture (farm one in Scotland; n = 136 comprising of 74 ewes and 62 rams, and farm two in Wales; n = 72 comprising of 40 ewes and 32 rams). Twelve lambs were hand-reared, but the majority were reared as singletons (n = 139) or twins (n = 57). Of the 208 lambs, 143 had known TM-QTL genotypes: 40 (14 rams and 26 ewes) were non-carriers ($+^S/+^D$), 17 (8 rams and 9 ewes) inherited a copy of the allele (TM) from their dam ($+^S/TM^D$), 53 (23 rams and 30 ewes) inherited a copy from their sire ($TM^S/+^D$) and 33 (14 rams and 19 ewes) inherited a copy from both their sire and dam (TM^S/TM^D). TM-QTL genotype was determined using four microsatellite markers on ovine chromosome 18 by *Pfizer Genetics*, New Zealand, as described by

Macfarlane *et al.* (2009). Growth rate for each lamb was calculated from birth weight and pre-slaughter weight (un-fasted) recorded on-farm. Procedures involving animals were approved by the SAC animal ethics committee and were performed under UK Home Office licence, following the regulations of the Animals (Scientific Procedures) Act 1986.

5.2.2 Abattoir and processing protocol

Lambs were slaughtered at a mean age of 144 days (range 126 to 155 days) and mean hot carcase weight (HCW) of 15.1 kg (range 8 to 25 kg, unadjusted for rearing rank and sire effects) on a single day in a commercial abattoir and were subjected to electrical stimulation (825 volts, 14 Hertz for 20 seconds) at approximately 40 minutes post mortem. Dressing-out percentage (DO%) was the HCW as a percentage of pre-slaughter weight (un-fasted). For logistical reasons carcases were chilled for seven (41 ewes and 28 rams), eight (42 ewes and 39 rams), or nine (31 ewes and 27 rams) days before butchering into joints. On the day of butchering, LL and SM were removed from the right hand side of the carcase, ultimate pH (pH_{ult}) was recorded at ambient temperature on all SM (n = 208) samples and on a subset of LL samples (n = 132, from days eight and nine only). The subset of samples where LL pH_{ult} was measured came from the left hand side of the carcase. A temperature compensated Testo 205 pH meter (Testo AG, Lenzkirch, Germany) was used after calibrating in pH4 and pH7 buffer solutions at ambient temperature. The right LL samples were vacuum packaged, frozen at –30°C and sent to Bristol University for meat quality assessment. A freshly exposed surface of SM was allowed to bloom for 45 minutes at ambient temperature before colour measurements (L*, a* and b*) were recorded using a Minolta CR-410, D65 illuminant, 2° standard observer calibrated with a CR-A44 white calibration plate. Following the colour measurement, all SM samples were frozen at –30°C in sealed plastic bags for subsequent analyses at SAC, Edinburgh.

5.2.3 Meat quality assessment of M. longissimus lumborum

For the textural measures, samples were cooked from frozen in 14 batches (eight to 24 per batch, balanced for sex) to an internal temperature of 78°C in a water bath pre-heated to 80°C, before being cooled rapidly on ice and held overnight at 4°C (Taylor *et*

al. 1995). A Stable Micro systems texture analyser (TA.XT2, Blackdown Rural Industries, Surrey, UK) fitted with Volodkevitch-type jaws was used to assess the toughness (Vincent and Lillford 1991). Ten 10 x 10 x 20mm cores were prepared from each LL sample so the muscle fibres ran longitudinally, enabling a bite-like test to be performed perpendicular to the fibre direction. The mean peak force (PF) from the ten sub-samples formed the final measures of toughness LL PF. For each sample the percentage moisture content in the right LL (Moist%) was determined by freeze-drying a small sample for 72 hours, followed by vacuum oven drying at 80°C for four hours and re-weighing. This freeze-dried sample was then used for determination of intramuscular fat percentage (IMF%) using petroleum ether (B.P. 40-60°C) as the solvent in a modified Soxhlet extraction (Cameron *et al.* 1999).

5.2.4 Meat quality assessment of M. semimembranosus

Samples of SM were later defrosted in 17 batches balanced for sex (ranging between 10 and 16 samples per batch) for 24 hours at 3°C. A sample of SM (average weight = 165 g, range = 64-292 g) was weighed prior to cooking in a polythene bag for 90 minutes in a water bath pre-heated to 70°C. After cooking, fluid was drained off and samples stored overnight at 3°C. Cooking loss (CL%) was calculated as the weight loss during cooking (after dabbing dry with a paper towel) as a percentage of the raw weight. Twelve shears (2/core), perpendicular to the fibre axis were performed on six cores with a 13 x 13 mm cross section using a square Warner-Bratzler blade (Purchas and Aungsupakorn 1993) fitted to a Lloyd texture analyser (Lloyd Instruments, UK) with a cross head speed of 230mm/min. The mean peak shear force (WBSF) was calculated from the 12 sub-samples.

5.2.5 Statistical methods

Variance components were estimated with the restricted maximum likelihood (REML) method using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). For each response variable, observations > 3 SD from the mean were excluded from the analysis on the basis that they were possible outliers; the number of observations included for each trait is listed in Table 5.1. Terms included in the linear mixed models used to determine the effects of sex on each trait are listed in Table 5.1. SM pH_{ult} was tested for

significance as both a linear and quadratic covariate in the models for SM traits and was retained in L*, a* and b* ($P < 0.05$) (not shown in Table 5.1). A cooking batch effect was also tested for SM WBSF and LL PF, but was not statistically significant. Apart from the sex effect, all other effects with a statistical significance of $P > 0.09$ were excluded from the models (Table 5.1). LL pH_{ult} was tested as both a linear and quadratic covariate on LL PF and Moist%, but was excluded because it was not statistically significant. No statistically significant ($P < 0.05$) interactions were found between any terms (including HCW) and interactions were consequently excluded from the models. Pre-cooked weight was tested as an additional covariate on SM WBSF and CL% and was retained for CL% only where there was a significant effect ($P < 0.001$). Age at slaughter was also tested and excluded from all models on the basis that it was not statistically significant. Coefficients of determination (R^2) and residual standard deviations (RSD) were generated by regressing the predicted values obtained from the mixed models against the observed values for each trait using the REG procedure of SAS. LL PF and SM WBSF values were log transformed (log_n) to ensure a normal distribution prior to analysis in the mixed models; so least-squares (L-S) means were back transformed to the original scale to simplify interpretation. To analyse the effects of TM-QTL on instrumental meat quality traits of SM, analysis was restricted to the 143 lambs with known TM-QTL genotype. A sex by genotype interaction was included in the model to test whether genotype effects differed between sexes. Terms included in the model for each trait are given in Table 5.2. Pair-wise comparisons of L-S means of MQ traits for the four TM-QTL genotypes and two sexes were estimated using t-tests (Bonferroni-adjusted for multiple comparisons). Pearson correlation coefficients (un-adjusted for any other terms in the mixed model) were derived between pairs of traits, using the CORR procedure of SAS. The Fisher's Z test was used to test pairs of correlation coefficients for statistically significant differences between the sexes (Kenny 1987).

5.3 Results and discussion

5.3.1 Lamb performance traits

Ram lambs had 5% higher growth rates ($P < 0.05$) and 4% higher pre-slaughter weights ($P < 0.05$) than ewe lambs (Table 5.1). Ewe lambs had a 3.2% higher DO% than rams

($P < 0.001$) possibly due to increased carcase fatness (data not shown), but there was no significant sex effect on HCW or age at slaughter (Table 5.1). The higher growth rates ($P < 0.05$) and lower DO% ($P < 0.01$) observed in ram lambs are in agreement with previous findings (Lee *et al.* 1990; Dransfield *et al.* 1990; Johnson *et al.* 2005; Okeudo and Moss 2008).

5.3.2 Meat quality of M. longissimus lumborum

The LL of ram lambs had a 13.3% higher shear force values than ewe lambs in terms of PF ($P < 0.001$) (Table 5.1). This finding corroborates the findings of Johnson *et al*. (2005) who reported that LL from pasture-fed Texel-cross ram lambs of a similar HCW to those in the current study was 14.5% tougher than that from ewe lambs using a modified Warner-Bratzler shear force instrument. The current results are also in agreement with those of Wojtysiak *et al*. (2010) who reported that ram LL had a higher shear force than ewe LL. There is further agreement between the current results and results of Lind *et al*. (2011) who assessed the sensory parameters of six-day aged LL from ram and ewe lambs finished on pasture for 24 days prior to slaughter. Lind *et al*. (2011) found that sensory tenderness in six day aged LL from ewe lambs was significantly more acceptable to consumers than the ram lamb samples, but lambs in that experiment were slaughtered at a higher live weight (> 40 kg) than lambs in the current analysis. The difference between sexes in terms of LL shear force in the current results is contrary to the results of Lambe *et al*. (2010b) who reported no significant sex effect on LL shear force using the same lab and the same method (Volodkevitch-type). Navajas *et al*. (2008) investigated the sex effect on LL sensory parameters from pasture-fed lambs of two different breeds slaughtered at an average age of 139 days. Panellists awarded higher overall liking scores to LL from ewe lambs, but made no distinction between the sexes for LL toughness (Navajas *et al.* 2008).

Placing the sex effects on LL shear force into a consumer context is difficult because consumer sensory appraisals are subjective and encompass a range of other sensory parameters including juiciness and flavour (Thompson 2004). Furthermore, there are few experiments that have correlated Volodkevitch shear force to consumer sensory panel scores for lamb meat. A subset ($n = 40$) of left LL muscles from lambs used in the

current analysis were subjected to a consumer taste test by Lambe *et al*. (2011). A low, but significant negative correlation between LL shear force and panel texture score (r = –0.39) was reported, although the taste panel consisted of very few panellists and 20% of the loins were only evaluated by a single assessor (Lambe *et al.* 2011). The relationship between different shear force instruments may not be linear across the entire range of toughness encountered in lamb meat (Hopkins *et al.* 2011). Therefore inferring consumer tenderness thresholds based on one shear force instrument may not be fully applicable to other shear force tests. Lambe *et al*. (2010b), (2011) opted for a threshold value of 5.5 kgF, above which they speculated there may be some adverse consumer reaction to lamb toughness. Other researchers have proposed that a toughness range 5.0-7.9 kgF may be appropriate for the MIRINZ bite test (Bickerstaffe *et al.* 2001) or lower thresholds of 49 N (5.0 kgF), 40 N (4.1 kgF) or 27 N (2.75 kgF) which may be appropriate for the Warner-Bratzler test (Shorthose *et al.* 1986; Hopkins *et al.* 2006). The L-S means for LL shear force presented here (2.94 kgF for ewes and 3.39 kgF for rams) are towards the lower end of this range which suggests that the sex effect is not likely to pose a major problem for LL toughness where meat has been electrically stimulated and sufficiently aged. Considering the absolute LL shear force values in the current analysis, 15 lambs had shear force values above 5.5 kgF, of which four were ewes and 11 were rams. The average HCW of lambs slaughtered in UK abattoirs between December 2008 and December 2011 was 18.9 kg (Anon 2012b). Lambs used in the current experiment were slaughtered at an average HCW of 15.1 kg so were somewhat lighter than lambs finished under normal commercial practice. Previous research has shown gender effects on lamb meat toughness appear to be minimal before rams become sexually mature (Young *et al.* 2006), although their longitudinal experiment focused on rams and castrated males rather than females.

The level of LL IMF% found in the Texel lambs was similar to the 1.33% reported for purebred Texels by Navajas *et al*. (2008) and 1.60% reported by Lambe *et al*. (2009a). The levels of IMF% in the Texel breed is relatively low compared to other breeds (Fisher *et al.* 2000; Hopkins *et al.* 2006; Warner *et al.* 2010). In the UK, the average IMF% of *M. longissimus* in lamb chops purchased at supermarkets was 3.20% (Angood *et al.* 2008), but it is of note that these lambs are mostly crossbred lambs and not purebred Texels. Hopkins *et al*. (2006) proposed that in order for lamb meat eating quality to be 'better than every day' in Australia, a minimum of 5.0% IMF is required,

although IMF% only explained 3% of the overall liking in that experiment. Whether UK consumers require 5.0% IMF for 'better than every day" lamb quality is not known and country differences in perception are not unusual.

The contribution of IMF% to lamb toughness is not fully understood, but the findings of Warner *et al*. (2010) supported a hypothesis that IMF% affects lamb toughness by altering meat structure rather than through influencing the meat ageing process. In the current results LL moisture content was significantly higher and IMF% significantly lower in ram lambs ($P < 0.001$). Testing IMF% or moisture content individually as additional covariates in the LL shear force model explained a small amount of additional variation in shear force (R^2% = 38, RSD = 1.3 and R^2% = 37, RSD = 1.3 respectively for IMF% ($P = 0.002$) and moisture content ($P = 0.02$) respectively). The sex effect on LL shear force was reduced, but remained significant ($P = 0.02$) after inclusion of these covariates, which rejects the hypothesis that the sex effect on LL shear force is solely due to lower IMF% or higher moisture content in ram LL.

Due to the fact that the LL shear force values were low in the current results, it seems likely that other factors such as the lower levels of insoluble collagen associated with a growth rate > 250 grams per day (Sylvestre *et al.* 2002) may compensate for the low levels of IMF% in some instances. Unfortunately the solubility of collagen was not measured in the current analysis and the growth rate of the lambs was much less than 250 grams per day (Table 5.1). For the subset of LL where pH_{ult} was available, ewes had a higher pH_{ult} than ram lambs ($P = 0.01$), which is in contrast to Johnson *et al*. (2005) who found LL from ram lambs to have significantly higher pH_{ult} than ewe LL and that a curvilinear relationship existed between LL shear values and pH_{ult}. Similarly, Bain *et al.* (2009) reported pH_{ult} from ram lambs was significantly higher than that of ewe lambs, although the authors concluded that the amount of variation accounted for by sex in that experiment was very low. In the current results, one LL pH_{ult} value from a ewe lamb was higher than pH = 6.0 and no evidence was found for a curvilinear relationship between pH_{ult} and LL shear force. Furthermore, pH_{ult} was not a significant predictor of LL shear force or moisture content. Lambe *et al*. (2009b) reported that pH_{ult} was not significantly correlated with sensory scores of Texel lamb LL toughness, juiciness or flavour. Hopkins *et al*. (2006) also found that pH_{ult} was not a significant predictor of LL sensory traits.

Table 5.1 Least-squares means showing sex effects on lamb growth, and carcase or meat quality parameters, together with the significance of other terms used in the statistical models.

Trait	ewe lambs		ram lambs		Effects[c] (*P* values)						R^2 (%) (RSD)[d]
Animal	*n*[a]	L-S mean[b]	*n*[a]	L-S mean[b]	SEX	RR	F	HCW	AGE	SIRE	
Growth rate (g/day^{-1})	114	184	94	194	0.03	< 0.001	ns	-	-	0.009	18.8 (33.3)
Pre-slaughter weight (kg)	114	30.4	94	31.7	0.04	< 0.001	0.05	-	-	< 0.001	26.4 (4.65)
Slaughter age (days)	114	141	94	141	0.58	ns	< 0.001	-	-	< 0.001	72.9 (3.9)
Carcase											
Dressing out (%)	114	48.08	94	46.57	< 0.001	ns	< 0.001	< 0.001	-	ns	51.7 (2.53)
Hot carcase weight (kg)	114	14.63	94	14.91	0.49	< 0.001	ns	-	-	< 0.001	22.0 (2.77)
M. longissimus lumborum (LL)[e]											
PF (kgF)	114	2.94	92	3.39	< 0.001	< 0.001	< 0.001	< 0.001	0.01	0.05	35.1 (0.27)[g]
Moist%	110	74.97	93	75.68	< 0.001	< 0.001	ns	< 0.001	ns	ns	27.3 (0.79)
IMF%	109	1.49	94	1.13	< 0.001	ns	ns	< 0.001	ns	ns	43.5 (0.42)
pH_{ult}	67	5.63	63	5.59	0.006	< 0.001	ns	0.003	ns	ns	20.8 (0.08)
M. semimembranosus (SM)[f]											
WBSF (kgF)	114	4.29	91	4.70	< 0.001	ns	ns	Ns	0.005	ns	10.8 (0.18)[g]
L*	113	43.13	90	44.64	< 0.001	< 0.001	ns	< 0.001	ns	ns	33.5 (1.52)
a*	114	23.37	92	23.19	0.16	< 0.001	< 0.001	< 0.001	< 0.001	0.008	55.4 (0.83)
b*	112	9.59	93	9.92	0.02	0.03	ns	Ns	ns	ns	18.1 (1.00)
pH_{ult}	108	5.58	93	5.58	0.95	0.02	0.05	Ns	0.009	0.07	17.3 (0.05)
CL (%)	112	30.82	92	32.36	< 0.001	ns	ns	0.03	ns	ns	53.7 (1.89)

[a] Number of ewe and ram lambs retained in the analysis for determining the sex effect after removal of records > 3 SD from the mean.
[b] Least-squares means for ewes and rams adjusted for other effects (the significance of the difference between ewes and rams is the SEX effect).
[c] Effects of terms included in the models (ns = tested but not statistically significant and not included, a dash (-) indicates that the term was not used in the model).
RR = Rearing Rank (single, twin or hand-reared, fixed effect), F = Farm (*n* = 2, fixed effect), HCW = Hot carcase weight (included as a covariate, AGE = carcase aging time (7, 8 or 9d, fixed effect), and SIRE (*n* = 7, random effect), Additional effects: Growth rate was corrected for birth weight (covariate), SM CL% had a batch effect (*n* = 17, fixed effect) and uncooked sample weight (covariate) included. SM pH_{ult} was included as both linear and quadratic covariates for SM colour traits (*P* values were equivalent for both linear and quadratic terms). The significance for these additional terms are given in the statistical methods (Section 5.2.5).
[d] Coefficient of determination (R^2%) and residual standard deviation (RSD).
[e] Within *M. longissimus lumborum*: PF = peak force (kgF), Moist% = moisture content, IMF% = intramuscular fat percentage, pH_{ult} = ultimate pH.
[f] Within *M. semimembranosus*: WBSF = Warner-Bratzler peak shear force (kgF), L* = lightness, a* = redness, b* = yellowness, pH_{ult} = ultimate pH, CL = cooking loss (%).
[g] RSD values for LL PF and SM WBSF are on the $\log_n$ scale.

5.3.3 Meat quality of *M. semimembranosus*

There are relatively few reports comparing SM meat quality between ram and ewe lambs (Pommier *et al.* 1989; Johnson *et al.* 2005; Navajas *et al.* 2008). In this study, SM samples from ewe lambs had lower shear force values compared to ram SM samples ($P < 0.001$), and a lower CL% ($P < 0.001$), lower L* values ($P < 0.001$) and lower b* values ($P = 0.02$), but a* and pH_{ult} were similar (Table 5.1). The finding that SM shear force was 8.6% higher in rams is in agreement with the results of Johnson *et al*. (2005) who reported 11.7% higher shear force values in rams using the same Warner-Bratzler protocol (but on a different instrument). Johnson *et al*. (2005) and Hopkins *et al*. (2007) obtained much higher SM shear force values than those in the current experiment probably because their samples were only aged for one day before freezing and subsequent shear force assessment. Pommier *et al*. (1989) did not find any difference in SM shear force between 48 Romanov ewe and ram lambs and Navajas *et al*. (2008) reported that there were no significant differences between the sexes in the sensory attributes of SM from Texel lambs. In contrast to Johnson *et al*. (2005), there were no significant differences in pH_{ult} between ewes and rams, and there was no evidence to support a curvilinear relationship between shear force and pH_{ult} or CL% and pH_{ult} in the current results. Of the six observations deemed possible outliers for pH_{ult}, all were females, five were above $pH_{ult} = 5.77$ and the lowest reading was $pH_{ult} = 5.35$. Including outliers did not result in a sex effect on pH_{ult} or a curvilinear relationship between SM pH_{ult}, shear force or CL% (not shown). When investigating SM colour parameters there was evidence of a weak curvilinear relationship between pH_{ult} and lightness, redness and yellowness, corroborating the previous findings reported by Johnson *et al*. (2005). The colour values of SM muscle in the current experiment were higher than those reported by Johnson *et al*. (2005), which could be due to any number of production, processing or sampling factors or may be due to differences between Minolta Chroma meters (Kerr and Hopkins 2010). The predicted means for L* and a* observed in the current results using an open-type Minolta fitting suggest that approximately 95% of Australian consumers would find the colour of SM acceptable (Khliji *et al.* 2010). The correspondence of this threshold to UK consumer preferences is not known.

5.3.4 Effects of TM-QTL genotype on M. semimembranosus quality

The magnitude of effects in the restricted dataset (Table 5.2) was similar to the overall dataset (Table 5.1) although there was no evidence of a curvilinear relationship between CL, a* or b* and pH_{ult} in the full dataset (Table 5.1). There were no statistically significant TM-QTL genotype effects on instrumental meat quality parameters of SM within sexes, and there were no significant genotype by sex interactions (Table 5.2). The lack of an effect in SM is consistent with the findings of Lambe *et al.* (2011) who reported that TM-QTL genotype had no statistically significant effects on instrumental meat quality parameters of LL and *M. vastus lateralis* of the same pure-bred Texel lambs used in the current analysis. There is further consistency in that the TM-QTL growth effects were restricted to the LL (Macfarlane *et al.* 2009).

Table 5.2 Least-squares means for those lambs of known genotype showing the effects of TM-QTL genotype and sex and their interaction on instrumental meat quality parameters of *M. semimembranosus*.

TM-QTL Genotype	Number[u]		Meat quality parameters[v]											
			WBSF (kgF)		L*		a*		b*		pH_{ult}		CL (%)	
	ram	ewe	Ram	ewe	ram	ewe	ram	ewe	ram	ewe	ram	ewe	ram	ewe
$+^S/+^D$	14	26	4.72^{ab}	4.16^{b}	44.11^{ab}	$*42.68^{bc}$	23.00^{a}	23.13^{a}	9.63^{a}	$*9.33^{a}$	5.56^{a}	$*5.59^{a}$	32.61^{ab}	30.53^{b}
$+^S/TM^D$	8	9	5.33^{a}	4.18^{ab}	44.47^{a}	43.73^{abc}	23.06^{a}	22.92^{a}	9.77^{a}	$*9.01^{a}$	5.56^{a}	$*5.58^{a}$	32.87^{a}	30.79^{ab}
$TM^S/+^D$	23	30	4.53^{ab}	4.35^{ab}	$**44.91^{a}$	42.22^{bc}	$*22.73^{a}$	23.07^{a}	9.68^{a}	9.30^{a}	5.58^{a}	$**5.56^{a}$	$*32.62^{a}$	31.09^{b}
TM^S/TM^D	14	19	4.54^{ab}	4.26^{ab}	$*44.70^{a}$	42.32^{c}	$*22.99^{a}$	23.08^{a}	9.75^{a}	9.02^{a}	5.57^{a}	5.57^{a}	32.09^{ab}	30.17^{b}
Average SED[w] (range)			1.07 (1.05-1.09)		0.53 (0.38-0.74)		0.32 (0.23-0.43)		0.37 (0.27-0.50)		0.02 (0.01-0.03)		0.74 (0.55-1.00)	

Effects[x]	*P* value	*P* value	*P* value	*P* value	*P* value	*P* value
Sex (*n* = 2, fixed)	< 0.001	< 0.001	0.51	0.006	0.64	< 0.001
TM-QTL Genotype (fixed, *n* = 4)	0.58	0.09	0.83	0.97	0.92	0.43
Sex*Genotype (fixed, *n* = 8)	0.22	0.28	0.77	0.64	0.11	0.92
Aging time (fixed, *n* = 3)	0.05	ns	< 0.001	< 0.001	ns	ns
Farm (fixed, *n* = 2)	ns	ns	< 0.001	ns	ns	ns
Rearing rank (fixed, *n* = 3)	0.34	0.003	< 0.001	< 0.001	0.02	ns
Batch (fixed, *n* = 14)	ns	-	-	-	-	ns
Hot carcase weight (covariate)	0.07	< 0.001	< 0.001	0.007	ns	< 0.001
pH_{ult}	ns	0.001	ns	ns	-	0.005
pH_{ult}^2	ns	0.001	ns	ns	-	0.005
Sire (random, *n* = 8)	ns	ns	ns	ns	0.007	ns
R^2 (%) (RSD)[y]	14.1 (0.17)[z]	47.9 (1.32)	60.5 (0.80)	33.4 (0.92)	21.9 (0.05)	34.8 (1.98)

Least-squares means within each trait sharing a character in their superscript (a,b,c) are not significantly different ($P > 0.05$), * represents the number of records excluded (* = 1, ** = 2) on the basis that they were outliers > 3 SD from the mean of the full (*n* = 208) dataset.

[u] Number of ewe and ram lambs with each TM-QTL genotype.

[v] WBSF = Warner-Bratzler peak shear force (kgF), L* = lightness, a* = redness, b* = yellowness, pH_{ult} = ultimate pH, CL = cooking loss (%).

[w] Average standard error of the difference between predicted means.

[x] Effects (*P* values) of terms included in the models, the type of effect (fixed, covariate or random) and number of levels for each model term are given in parentheses. ns = tested but not significant and not included, a dash (-) indicates that the term was not tested or included in the model.

[y] Coefficient of determination (R^2%) and residual standard deviation (RSD).

[z] RSD values for WBSF are on the $\log_n$ scale.

5.3.5 Correlations between traits

Selected correlations between HCW and meat quality traits and correlations between traits within each muscle are presented in Table 5.3. It is of note that different measurement methods for meat quality were applied to different muscles. As a result, correlations between muscles are confounded with the methods and are therefore invalid. There was a low negative correlation between HCW and SM CL% which may be due to smaller carcases yielding smaller samples of SM which would have a higher surface area to volume ratio resulting in greater cooking loss. There was a low negative correlation between HCW and SM L*, which is in agreement with previous findings (Caneque *et al.* 2001; Wojtysiak *et al.* 2010). There was a positive but low correlation between HCW and SM a* possibly because myoglobin concentration increases with lamb age (Hopkins *et al.* 2007; Kim *et al.* 2012) although the range of ages in the current analysis was low by comparison to those of Hopkins *et al*. (2007) and Kim *et al*. (2012).

Table 5.3 Pair-wise phenotypic correlation coefficients for hot carcase weight and lamb meat quality traits of *M. semimembranosus* (SM) and *M. longissimus lumborum* (LL) based on raw data for 84 entire ram and 100 ewe lambs after removal of records > 3 SD from the mean.

M. semimembranosus						
Trait[a]	HCW	WBSF	L*	a*	b*	pH_{ult}
WBSF	–0.05					
L*	**–0.33**	**0.18**				
a*	**0.42**	0.00	**–0.15**			
b*	0.11	0.11	**0.39**	**0.73**		
pH_{ult}	0.10	0.02	0.05	–0.12	**–0.23**	
CL%	**–0.40**	**0.21**	**0.54**	–0.14	**0.15**	0.11
M. longissimus lumborum						
Trait[a]	HCW	PF	Moist%	IMF%		
PF	**–0.22**					
Moist%	**–0.25**	**0.30**				
IMF%	**0.57**	**–0.36**	**–0.58**			
pH_{ult}[b]	–0.15	0.10	**0.20**	–0.13		

Correlation coefficients in bold are significantly different from zero ($P < 0.05$).
[a] HCW = Hot carcase weight (kg), Within *M. semimembranosus*: WBSF = Warner-Bratzler peak shear force, L* = lightness, a* = redness, b* = yellowness, pH_{ult} = ultimate pH, CL% = cooking loss. Within *M. longissimus lumborum*: PF = peak force, Moist% = moisture content, IMF% = intramuscular fat percentage, pH_{ult} = ultimate pH. [b] Correlation based on $n = 57$ rams $n = 58$ ewes for LL pH_{ult}.

The correlation between HCW and IMF% in LL was moderately positive, but correlations were significantly higher ($P < 0.001$) in ram lambs ($r = 0.72$) than ewe lambs ($r = 0.38$) (results not shown). The positive correlation between HCW and IMF% is consistent with previous reports (Pethick *et al.* 2005; Hopkins *et al.* 2007). There was little if any correlation between LL pH_{ult} and SM pH_{ult} after excluding outliers ($n = 6$).

5.3.6 Future directions for lamb meat quality analysis

Further research is needed to determine whether the effects of gender and genotype would alter if meat was aged for a shorter period of time or if lambs were slaughtered at a heavier live weight. More research is also required to determine the relationship between instrumental meat quality and sensory evaluation of lamb meat eating quality. Existing relationships between instrumental meat eating quality and sensory scores are highly variable and not directly transferable between experiments because assessment methods vary and eating quality preferences differ between countries (Sañudo *et al.* 2007).

5.4 Summary and conclusions

1. The shear force of LL and SM was significantly higher in ram lambs, although shear values were relatively low overall, possibly because carcases were aged for between seven and nine days.

2. The presence of one or two copies of the TM-QTL allele appeared to have no statistically significant effects on lamb meat quality.

3. An acceptability threshold for instrumentally derived toughness for lamb does not currently exist for UK consumers so toughness acceptability thresholds are somewhat speculative.

4. Some correlations differed by sex, so correlations, when calculated over sexes should be interpreted with caution.

5. Finishing ram lambs to the specifications used in this experiment resulted in meat with relatively minor although statistically significant differences in meat quality relative to that from ewe lambs.

6. To further characterise effects of sex on lamb meat eating quality a sensory analysis is required on meat from lambs finished to average commercial specifications where the average HCW for the UK would be 18.9 kg.

6 Investigations into relationships between visible-near infrared (NIR) spectra and instrumental meat quality parameters in lamb *M. longissimus lumborum*, *M. semimembranosus* and *M. vastus lateralis*

Abstract

Current lamb carcase evaluation systems approximate saleable meat yield but there is no provision for meat eating quality. A carcase evaluation system that encourages producers to maximize saleable meat yield without monitoring the effects on meat quality may lead to poorer meat quality over time. Current methods for assessing lamb meat quality are expensive, time consuming, labour intensive and destructive rendering them unsuitable for carcase evaluation purposes. The aim of this experiment was to determine the ability of visible-near infrared (NIR) spectroscopy data collected on fresh (never-frozen) lamb *M. longissimus lumborum* (LL) to predict instrumental meat quality parameters of LL, *M. semimembranosus* (SM) and *M. vastus lateralis* (VL). NIR spectra collected under experimental conditions on the LL had a limited ability to predict Volodkevich shear force (R^2_{pred} = 29.1, SE_{pred} = 1.05 kgF) and models were unable to correctly identify all samples with shear force values > 5.5 kgF. Shear force of SM or VL could not be predicted with NIR spectra collected on LL, but promising results were obtained for SM lightness (R^2_{pred} = 60%, SE_{pred} = 1.34) and redness (R^2_{pred} = 54%, SE_{pred} = 0.92). The processing rate and variety of genders and genotypes encountered at a commercial lamb slaughter plants is likely to present significant challenges for the technology. Future efforts should investigate the relationship between NIR spectra and meat quality parameters taking these factors into account.

6.1 Introduction

The total value of a lamb carcase is determined by the yield of saleable meat and the eating quality of the meat. Lamb carcase evaluation systems based either on video image analysis or a visual assessment system aim to quantify the saleable meat yield, but they do not directly predict meat eating quality (Stanford *et al.* 1998; Rius-Vilarrasa *et al.* 2009). In order to maximize profits using the current carcase evaluation systems, lamb producers are encouraged to maximize saleable meat yield without considering the possible impacts of this on the eating quality of the meat. Producers may maximize saleable meat yield by either reducing the total carcase fat percentage or increasing the carcase muscle-to-bone ratio (Purchas *et al.* 2002b; Johnson *et al.* 2005). Both aims may be achieved through selective breeding for traits and possibly using additional molecular genetic information on certain markers and genetic polymorphisms such as the Texel muscling quantitative trait locus (TM-QTL) (Walling *et al.* 2004; Macfarlane *et al.* 2009). Detailed discussion on the effects of the TM-QTL on carcase and instrumental meat quality parameters was presented in Sections 5.1 and 5.3.4. Encouraging farmers to select for saleable meat yield may have detrimental effects on meat tenderness. For example, selecting for very lean pig carcases had profound effects on the muscle fibre composition of the pork, reducing the percentage of slow oxidative fibres and increasing the percentage fast glycolytic fibres which are associated with increased toughness in pork (Maltin *et al.* 2003). Indeed, slow oxidative fibres have also been associated with tenderness in lamb (Wojtysiak *et al.* 2010) and the percentage of slow fibres is lower in the *M. longissimus lumborum* (LL) in Texel lambs than in the Scottish Blackface lambs which are, regarding carcase quality, a less-improved breed (Bunger *et al.* 2009). Furthermore, selecting for leanness in lambs using lean terminal sire breeds might lead to a reduction in intramuscular fat percentage (IMF%) (Mortimer *et al.* 2010; Hopkins *et al.* 2011a). Hopkins *et al.* (2006) propose that 5% IMF is a target for 'good everyday' eating quality in lamb. The IMF% in purebred Texel lambs is low, ranging from 1.33% to 1.60% (Navajas *et al.* 2008; Lambe *et al.* 2009a; Lambe *et al.* 2011). Using a carcase evaluation system focused entirely on yield characteristics without any measure of meat quality parameters encourages producers to increase yield but this may come at the expense of meat eating quality.

Lamb meat eating quality has traditionally been determined either by using sensory panels (Vipond *et al.* 1995; Sañudo *et al.* 2000; Goodson *et al.* 2001; Safari *et al.* 2001) or instrumentally, using shear force as a proxy for tenderness or other measures of product quality such as ultimate pH, meat colour and cooking loss (Shorthose *et al.* 1986; Shackelford *et al.* 1997; Hopkins and Fogarty 1998; Shackelford *et al.* 2004; Hopkins *et al.* 2011b). All of these methods are time consuming and labour intensive and not adequate for integrated measurements in the slaughter-line, furthermore both sensory assessment and shear force tests result in destruction of the meat, and thus it is unlikely that they will be adopted for routine carcase evaluation.

Visible-near infrared (NIR) spectroscopy in combination with multivariate calibration and prediction phases has been identified as a suitable technology to predict meat quality parameters in a fast, non-destructive, safe and cost-effective manner (Osborne *et al.* 1993). These properties make NIR appealing for routine carcase evaluation; but there is still a lack of evidence regarding the performance of the technology for predicting lamb meat quality. The theory and performance of NIR to measure meat quality parameters is discussed in Section 2.7, and is the subject of three excellent reviews (Prevolnik *et al.* 2004; Prieto *et al.* 2009a; Weeranantanaphan *et al.* 2011).

In contrast to beef, where there are at least 20 published experiments on attempts to apply NIR to predict instrumental measures of meat quality (Table 2.15, Table 2.16 and Table 2.17), there are only two published experiments where NIR has been used to predict instrumental meat quality parameters in lamb (McGlone *et al.* 2005; Andrés *et al.* 2007). Thus while the determination of instrumental meat quality in beef and lamb has many similarities, there is little evidence to support the performance of NIR for predicting instrumental quality parameters in lamb.

Using NIR spectra collected on the LL, McGlone *et al.* (2005) developed calibration equations for predicting MIRINZ shear force values over a range of aging times (0, 8, 24 and 72 hours). McGlone *et al.* (2005) reported a coefficient of determination for the prediction (R^2_{pred}) values of 85% within 65 lambs of known background, but using this model on another 12 lambs of unknown background the R^2_{pred} reduced to 44% suggesting that the model was not robust. Andrés *et al.* (2007) reported the predictive ability (by cross validation as opposed to prediction on new samples) of NIR spectra

collected on samples of lamb LL that had been frozen and thawed to predict sensory parameters of lamb meat quality on a scale of 1-8. The coefficient of determination for the cross-validation (R^2_{cv}) that study was R^2_{cv} = 12.5% (SE_{cv} = 0.85) for texture, R^2_{cv} = 29.5 (SE_{cv} = 0.44) for juiciness, R^2_{cv} = 27.1 (SE_{cv} = 0.47) for flavour, R^2_{cv} = 4.8% (SE_{cv} = 0.44) for abnormal flavour and R^2_{cv} = 24.3, (SE_{cv} = 0.48) for overall liking. Furthermore, IMF% could be predicted with an R^2_{cv} = 79.4% (SE_{cv} = 0.41) and R^2_{cv} = 18.8 (SE_{cv} = 0.16) for pH at 24 hours post mortem.

Freezing and thawing is known to alter the NIR spectra of beef (Downey and Beauchêne 1997), it is possible that spectra from frozen lamb would also differ to spectra of fresh lamb. Furthermore, if NIR was to be used for carcase evaluation purposes, the models would need to predict meat quality from fresh lamb. Neither McGlone *et al.* (2005) nor Andrés *et al.* (2007) investigated the ability of NIR to predict the quality of additional muscles, so it is not known whether NIR collected on the LL can be used to predict the tenderness of other muscles. Lambe *et al.* (2010b), (2011) reported low correlations between LL and *M. vastus lateralis* for Volodkevitch shear force on 166 lambs (r = 0.12) and 208 lambs (r = 0.22). As a consequence, it cannot be assumed that LL shear force is indicative of the shear force in other muscles in the lamb carcase.

The aim of the current experiment was to:

- Determine the ability of NIR spectroscopy data collected on fresh (never-frozen) lamb LL to predict instrumental meat quality parameters of *M. longissimus lumborum*, *M. semimembranosus* and *M. vastus lateralis*.

6.2 Materials and methods

6.2.1 Animals and meat quality measurements

Details of the 208 lambs used in the analysis are given in Section 5.2.1. The abattoir and processing protocol is given in Section 5.2.2. Meat quality assessment of LL is given in Section 5.2.3. The protocol for measuring Volodkevitch shear force for *M. vastus lateralis* (VL) was identical to that of LL. Meat quality assessment of *M. semimembranosus* (SM) is outlined in Section 5.2.4. The left LL was obtained from a subset (n = 169) of the lambs as outlined by (Lambe *et al.* 2011) for MIRINZ tenderometer assessment (Macfarlane and Marer 1966). Briefly, samples were suspended in polythene bags and cooked to an internal temperature of 75°C in a water bath pre-heated to 100°C. Samples were held at 2°C for 48hrs before ten shears perpendicular to the fibre axis were performed on separate 10mm by 10mm by 25mm cores (Bickerstaffe *et al.* 2001). In addition to the lightness (L*), redness (a*) and yellowness (b*) colour parameters recorded on the SM, hue angle (brownness) and chroma (saturation) were calculated using the formulas given in Hunt *et al.* (1991).

6.2.2 NIR spectra collection

After the LL was removed from the carcase at 7, 8 or 9 days post mortem, a 15 mm slice was taken from the anterior end of the muscle for NIR spectra collection. The freshly cut surface was allowed to bloom for two minutes (Shackelford *et al.* 2005). An ASD Labspec 5000 (ASD Inc., Boulder Colorado) NIR spectrometer fitted with a high-intensity contact probe (Figure 6.1) with a 10 mm spot size was operated using a laptop computer running the Indico Pro program (ASD Inc.). Ten replicate NIR spectra (350-2500 nm at 1 nm intervals) were collected by removing and replacing the scanning head on the meat surface between scans.

Figure 6.1 The ASD Labspec 5000 NIR spectrometer (left), the high intensity contact probe (centre) and application to a slice of lamb LL (right).

6.2.3 Pre-processing of NIR spectra

Spectra were recorded as absorbance log (1/Reflectance). Plotting all spectra revealed that regions at the extremes of the range (350-2500 nm) contained excessive noise (Figure 6.2). Removing these sections (350 to 499 nm and 1801-2500 nm) resulted in 500-1800 nm as the working spectra (Figure 6.3).

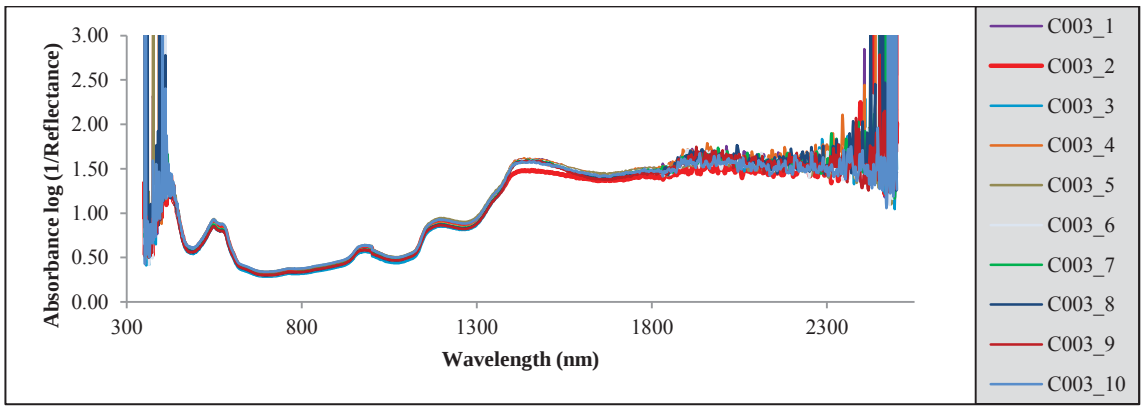

Figure 6.2 Ten replicate scans from one sample (C003) over the full range (350-2500 nm), excessive noise can be seen at the ends of the spectral region.

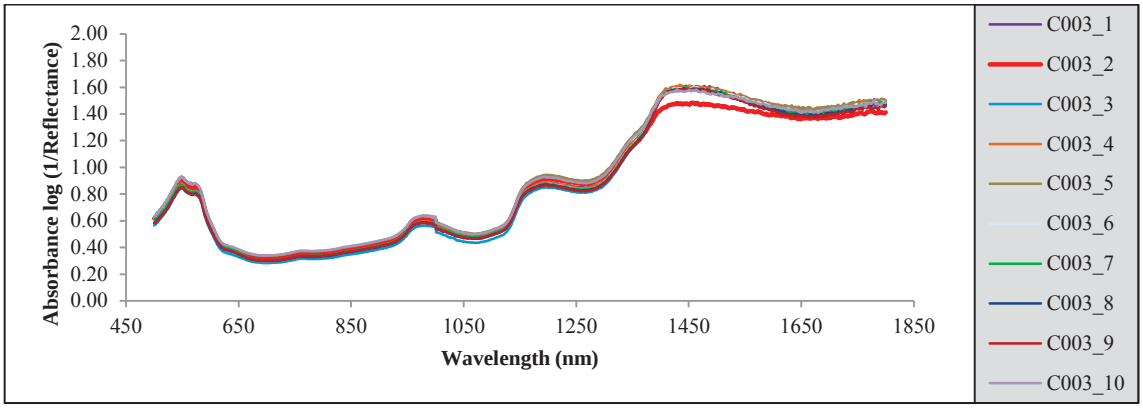

Figure 6.3 Working spectral range (500-1800 nm) after removing excessive noise at the upper and lower extremes.

The ten replicate working spectra (hereafter referred to as "spectra") for each sample were subject to an initial principal component analysis to detect outliers. A Hotelling T^2 ellipse with the critical test value of $\alpha = 0.25$ was fitted to the principal components scores plot between PC 1 and PC 2 using the Unscrambler (version 10.1) multivariate analysis software (Camo Software AS. Oslo, Norway) (Figure 6.4). Replicate spectra lying outside the Hotelling T^2 ellipse were deemed to be outliers (Krizsan *et al.* 2007). Such an approach could be used to determine the need to re-scan in an on-line application. On average, approximately 1 out of 10 replicate working spectra were sufficiently different from the remaining 9 to be deemed outliers. From Figure 6.4, it can be seen that C003_2 in this example was deemed an outlier, by looking at Figure 6.2 and Figure 6.3, it can be seen that the spectra of C003_2 (heavier red line) is noticeably different from the other 9 replicates. After removal of outliers, the median value at each wavelength for the remaining replicate spectra was calculated to form the final spectral reading for each meat sample.

Scatter effects resulting from interactions between light and structural properties of the sample (such as particles or droplets) (Osborne *et al.* 1993) were visualized by plotting each individual spectra against the average of all spectra (Geladi *et al.* 1985). Additive effects are seen as different y-axis offsets for different spectra while multiplicative scatter effects are seen as peak intensity dependant spread between different spectra (Esbensen *et al.* 2009). Mostly additive scatter effects were present in the median spectra (Figure 6.5), in order to resolve the scatter effects, several spectral pre-treatments were applied including; multiplicative scatter correction (MSC), baseline

correction and standard normal variate (SNV) transformation. The spectral transformations used for each parameter are listed in Table 6.2.

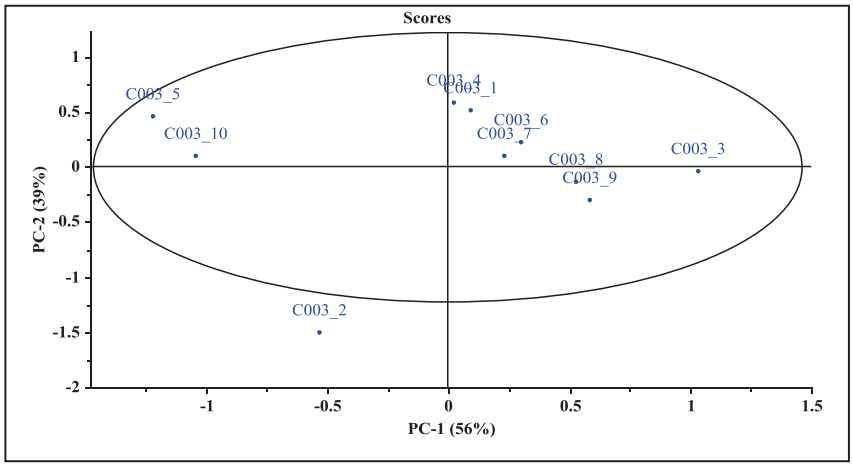

Figure 6.4 A Hotelling T^2 ellipse (α = 0.25) superimposed over the principal component analysis of the 10 replicate spectra. It can be seen that C003_2 is deemed an outlier using this criteria.

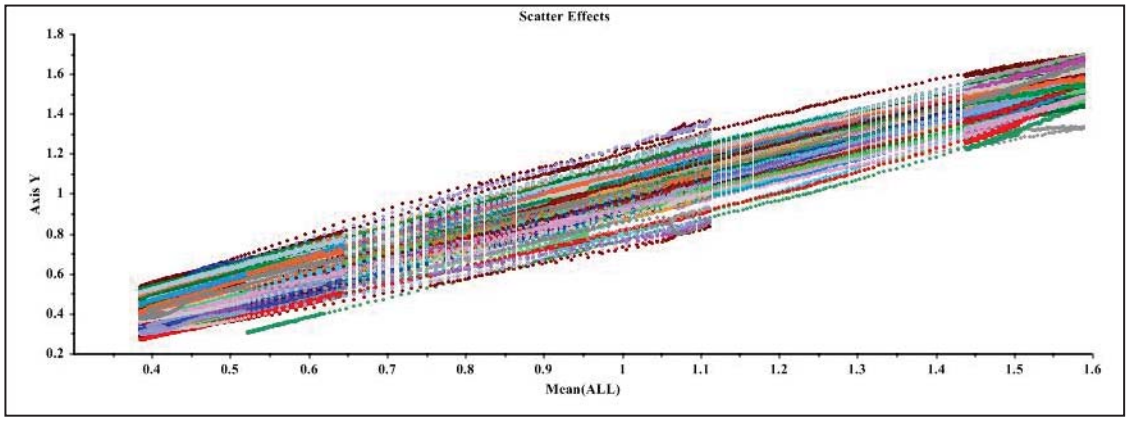

Figure 6.5 A plot of individual spectra against the average of all spectra, some additive scatter effects are present as can be seen by the apparent stacking of the spectra.

Details of the methods for each transformation are given in Esbensen *et al.* (2009). Plotting each spectra against the average of all spectra (Geladi *et al.* 1985) revealed the MSC, Baseline correction and SNV transformations had removed the scatter effects and improved the signal to noise ratio for Yellowness, Chroma, Hue, Cooking loss, shear force traits and pH_{ult}, but no pre-treatments were deemed necessary for lightness or redness.

6.2.4 Analysis of NIR data

In order to split samples into a calibration and prediction datasets, the samples were sorted in ascending order separately for each parameter and every fourth sample was assigned to the prediction dataset, with the intervening three samples being allocated to the calibration dataset as recommended by Williams (2001). As a result, the samples assigned to each dataset are dependant on the trait of interest, thus the prediction dataset is representative of the calibration dataset with a similar mean, standard deviations and range to the calibration dataset. The prediction dataset is only used for testing the model as recommended by Naes *et al.* (2002). The calibration model was then subject to full leave-one-out cross-validation where each sample is removed, predicted, and replaced in a sequential manner (Naes *et al.* 2002). Partial least squares regression type 1 was used for predicting instrumental meat quality traits on the three muscles using median NIR spectra (500-1800 nm) from the replicates that had not been rejected as outliers as explanatory variables. Outliers were handled as described in Section 4.2.7 on page 92.

6.3 Results and discussion

Descriptive statistics for the calibration and prediction dataset are shown in Table 6.1. The means are similar between the datasets yet there are some differences in the standard deviations (SD) between the data sets.

Table 6.1 Descriptive statistics for the calibration and prediction datasets.

	Calibration dataset				Prediction dataset			
Parameter	*n*	Mean	SD	Range	*n*	Mean	SD	Range
M. Longissimus lumborum								
Volodkevich shear force (kgF)	156	3.11	1.39	1.67-9.83	52	3.02	1.23	1.93-7.86
MIRINZ shear force (kgF)	126	3.73	1.47	2.00-9.44	43	3.81	1.60	1.84-10.68
Intramuscular fat (%)	153	1.36	0.68	0.37-6.17	51	1.31	0.56	0.37-2.78
Moisture content (%)	154	75.09	1.00	71.16-78.57	51	75.05	0.96	72.22-77.17
Ultimate pH (pH_{ult})	99	5.58	0.12	5.42-6.34	33	5.57	0.10	5.33-5.83
M. Semimembranosus								
Warner-Bratzler shear force (N)	156	45.61	11.36	24.28-110.29	51	45.18	9.34	30.65-79.73
Lightness (L*)	156	43.69	2.16	39.83-52.48	52	43.56	2.11	38.87-50.73
Redness (a*)	156	23.88	1.30	19.64-26.81	52	23.80	1.35	19.54-26.38
Yellowness (b*)	156	9.88	1.15	6.07-12.10	52	9.80	1.23	5.62-11.79
Chroma (C*)	156	25.85	1.51	21.82-29.16	52	25.76	1.60	20.62-28.93
Hue (H*)	156	22.43	1.94	15.59-28.82	52	22.28	2.04	14.79-27.20
Ultimate pH (pH_{ult})	156	5.56	0.07	5.42-5.93	51	5.56	0.06	5.45-5.81
Cooking loss (%)	154	31.57	2.61	25.29-40.63	51	31.47	2.55	25.65-37.96
M. vastus lateralis								
Volodkevich shear force (kgF)	156	2.91	0.56	1.77-4.95	52	2.88	0.54	1.77-4.70

6.3.1 Prediction of M. longissimus lumborum traits

The ability of NIR spectra collected on the LL surface to predict instrumental meat quality parameters on the same muscle is presented in Table 6.2. Due to the additive nature of the scatter effects (Figure 6.5), baseline correction and multiplicative scatter correction were the most effective transformations of the median spectra prior to the calibration and prediction phases as they reduced the amount of stacking and fanning respectively seen in Figure 6.5 (the scatter effects of the transformed spectra are not shown). Most published analyses report either the R^2 for the calibration (R^2_{cal}) or cross-validation (R^2_{cv}) and the standard error of the cross validation (SE_{cv}) as the final indicator of predictive ability, but very few report the R^2 for prediction (R^2_{pred}) or standard error of prediction (SE_{pred}) so comparison with previous reports on the basis of actual prediction performance is difficult. Because prediction ability is dependant on the variation in the raw data of the trait to be predicted, one method of comparison is to use the ratio of performance deviation (RPD) which is the standard deviation of the Y variable in the calibration dataset divided by SE_{cv} (RPD_{cv}) or preferably the standard deviation of the Y variable in the prediction dataset divided by the SE_{pred} (RPD_{pred}) (Cozzolino *et al.* 2000; Williams 2001; Prieto *et al.* 2009a; Agelet and Hurburgh 2010). An $RPD_{cv/pred}$ above 8 indicates the model is excellent and can be used with confidence where as $RPD_{cv/pred}$ values below 2.3 indicate a very poor model and application is not recommended, if the SE_{pred} is similar to the SD of the reference data (RPD ≈ 1.00), the instrument is not predicting the reference data. (Williams 2001).

The R^2_{cal} for Volodkevich shear force (R^2_{cal} = 25%) and MIRINZ shear force (R^2_{cal} = 25%) were identical (Table 6.2), and as expected, performance dropped in the cross-validation phase where the R^2_{cv} values were similar for Volodkevitch (R^2_{cv} = 21%, SE_{cv} = 1.06 kgF, RPD_{cv} = 1.31) and MIRINZ shear force (R^2_{cv} = 19%, SE_{cv} = 1.34 kgF, RPD_{cv} = 1.10). The calibration and cross-validation results MIRINZ shear force are shown in Figure 6.6. Two samples were removed from the prediction dataset for Volodkevich shear force, as the reference values were 9.16 and 9.83 kgF, which were >4 SD from the mean and could be justified as outliers in the Volodkevich shear force reference measure, if these sample was retained, the R^2_{cal} for Volodkevich shear force was 23%, SE_{pred} = 1.21 kgF).

Table 6.2 Performance of NIR calibration equations showing the coefficient of determination (R^2) and standard error (SE) for calibration, cross-validation and prediction phases for predicting instrumental meat quality parameters of lamb from spectra collected on *M. longissimus lumborum*. The calibration phase was performed on 70% of the data and models subsequently applied on the remaining 30% of the data to gauge predictive ability.

			Calibration			Cross-validation			Prediction			
Lamb meat quality parameter	Pre-treatment[a]	PC[b]	*n*[c]	R^2(%)	RMSE[d]	R^2(%)	SE	RPD[e]	*n*[f]	R^2(%)	SE	RPD[g]
M. Longissimus lumborum												
Volodkevich shear force (kgF)	Baseline	2	154	24.6	1.03	21.4	1.06	1.31	52	29.1	1.05	1.17
MIRINZ shear force (kgF)	Baseline	2	126	24.6	1.27	18.7	1.34	1.10	42	44.0	1.04	1.33
Intramuscular fat (%)	Baseline	7	151	68.5	0.31	57.4	0.36	1.88	51	64.9	0.33	1.68
Moisture content (%)	MSC	3	152	38.1	0.71	32.6	0.75	1.33	52	8.7	0.91	1.06
Ultimate pH (pH_{ult})	Baseline	5	96	31.7	0.08	13.3	0.09	1.37	33	0	0.10	1.00
M. Semimembranosus												
Warner-Bratzler shear force (N)	na	0	156	0	na[h]	na[h]	na[h]		51	na[h]	na	na
Lightness (L*)	none	3	156	49.7	1.53	46.7	1.59	1.36	52	59.7	1.34	1.57
Redness (a*)	none	6	156	56.7	0.85	46.7	0.95	1.36	52	53.9	0.92	1.47
Yellowness (b*)	MSC	6	153	49.1	0.80	30.0	0.95	1.20	51	36.0	0.96	1.26
Chroma (C*)	MSC	7	156	59.8	0.95	37.8	1.19	1.25	52	57.4	1.03	1.55
Hue (H*)	Baseline	4	156	24.1	1.69	12.2	1.83	1.06	52	29.3	1.71	1.19
Ultimate pH (pH_{ult})	MSC	0	156	0	na[h]	na[h]	na[h]	na[h]	51	na[h]	na	na
Cooking loss (%)	SNV	3	153	37.6	2.05	32.7	2.15	1.21	51	29.5	2.11	1.21
M. vastus lateralis												
Volodkevich shear force (kgF)	none	0	156	2.2	0.55	1.0	0.56	1.00	52	6	0.53	1.02

[a] Pre-treatments applied to the spectra prior to PLSR analysis and prediction, Baseline = Baseline correction, MSC = Multiplicative Scatter Correction and SNV = Standard Normal Variate.
[b] PC = number or principal components used in the regression.
[c] *n* = number of samples included in calibration and cross-validation phases.
[d] RMSE = root mean square error.
[e] RPD = ratio performance deviation is the SD of the Y variable in the calibration dataset (after removal of outliers) divided by the SE_{cv}.
[f] *n* = number of samples used for the prediction phase.
[g] RPD = ratio of performance deviation (SD of the prediction dataset divided by the SE of prediction).
[h] na = not available, where the calibration, cross-validation or prediction phase failed.

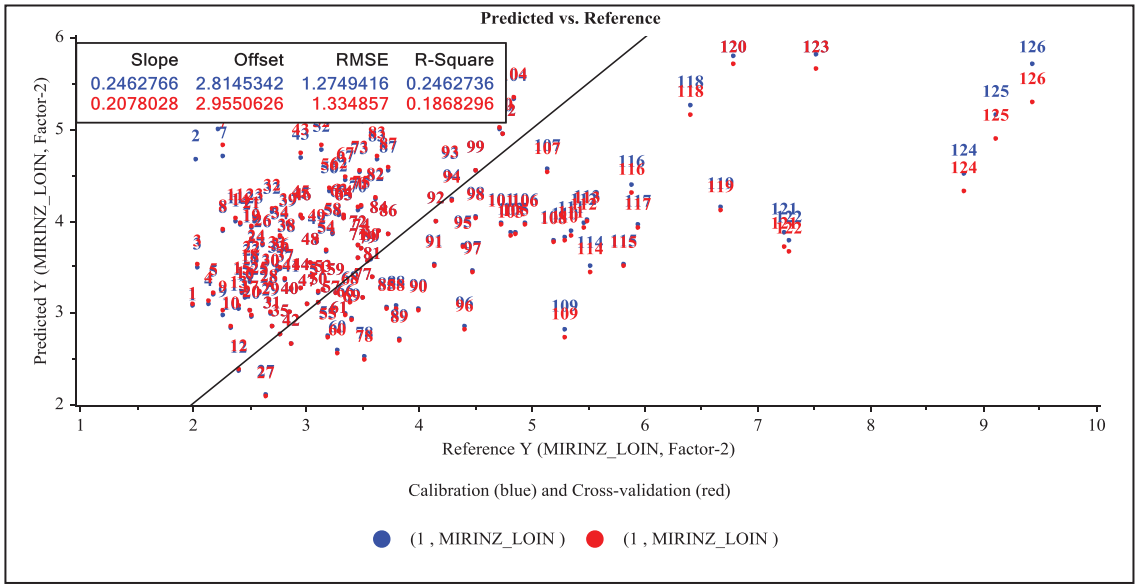

Figure 6.6 Calibration and cross-validation performance for MIRINZ shear force.

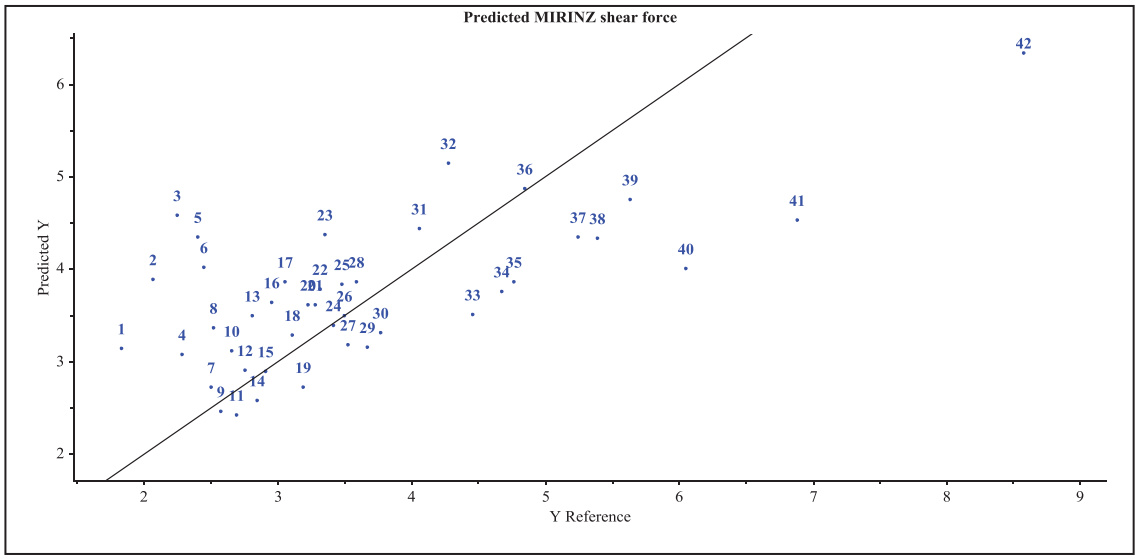

Figure 6.7 Prediction of MIRINZ shear force with NIR spectroscopy, R^2_{pred} and SE_{pred} are given in Table 6.2. Y Reference = actual MIRINZ shear force measurements of the prediction dataset, Predicted Y = predicted MIRINZ shear force values using NIR spectra.

Unexpectedly, the models performed better on the prediction dataset for both Volodkevitch shear force (R^2_{pred} = 29.1%, SE_{pred} = 1.05 kgF, RPD_{pred} = 1.17) and MIRINZ shear force (R^2_{pred} = 44%, SE_{pred} = 1.04, RPD_{pred} = 1.33) (Figure 6.7). After the exclusion of an extreme value (10.68 kgF) in the MIRINZ prediction dataset, the R^2_{pred} was the same as the R^2_{pred} = 44% reported by McGlone *et al.* (2005) who applied their model to predict MIRINZ shear force on an independent dataset of 12 lambs with unknown backgrounds. Retaining the outlier reduced the R^2_{pred} to 36%, SE_{pred} = 1.38

(data not shown). In the prediction dataset for Volodkevitch shear force, there were three samples that had shear force values > 5.5 kgF which has been identified as a toughness threshold above which there may be adverse consumer reaction (Lambe *et al.* 2011). The model was unable to identify these samples as having a shear force value > 5.5 kgF (results not shown). In the MIRINZ prediction dataset, four samples that had MIRINZ shear force values > 5.5 kgF, despite the encouraging performance, the model only correctly predicted one out of four samples that had a MIRINZ shear force value > 5.5 kgF, although of the remaining 38 samples with reference MIRINZ shear force values < 5.5 kgF, none were predicted above this value (results not shown). McGlone *et al.* (2005) reported much greater predictive ability of NIR spectra collected on intact LL from 65 lambs to predict MIRINZ shear force (R^2_{pred} = 85%, SE_{pred} = 12.2 N) within lambs of a known background, but they collected multiple spectra from the same meat at four different aging times post slaughter (0, 8, 24 and 72 hours post mortem) and the lamb was not aged beyond these aging times prior to MIRINZ assessment. McGlone *et al.* (2005) did not provide the standard deviation of the MIRINZ shear force for their prediction dataset so a comparison based on RPD could not be made. In the current experiment, spectra were collected at 7, 8 or 9 d post mortem, after which a greater amount of post-mortem proteolysis is likely to have occurred (Koohmaraie *et al.* 1991).

After removal of two outliers, a high R^2_{cal} was obtained for intramuscular fat percentage (IMF%) (R^2_{cal} = 69%, SE_{cv} = 0.36%), and the prediction performance was similar (R^2_{pred} = 65%, SE_{pred} = 0.33, RPD_{pred} = 1.68). Cozzolino *et al.* (2000) predicted IMF from spectra collected on intact LL samples with R^2_{cal} = 34.1% (SE_{cal} = 6.9 g/kg) and R^2_{cv} = 18.5% (SE_{cv} = 8.1 g/kg, RPD = 1.74_{cv}), they found that spectra collected on minced samples had a much higher predictive ability (R^2_{cv} = 70.6%, SE_{cv} = 4.7 g/kg, RPD_{cv} = 3.00). The same authors also reported the ability of NIR to predict IMF on minced samples from *M. infraspinatus* (R^2_{cv} = 19.6%, SE_{cv} = 12.30 g/kg, RPD_{cv} = 1.05), *M. supraspinatus* (R^2_{cv} = 54.7%, SE_{cv} = 7.41 g/kg, RPD_{cv} = 2.00), SM (R^2_{cv} = 45.0%, SE_{cv} = 5.13 g/kg, RPD_{cv} = 1.68), *M. semitendinosus* (R^2_{cv} = 51.6%, SE_{cv} = 7.08 g/kg, RPD_{cv} = 1.82) and *M. rectus femoris* (R^2_{cv} = 49.4%, SE_{cv} = 7.28 g/kg, RPD_{cv} = 2.12). Andrés *et al.* (2007) used NIR spectra collected on intact lamb LL to predict IMF% with an R^2_{cv} = 79% (SE_{cv} = 0.41%, RPD_{cv} = 2.19), but the pure-bred Texel lambs in the current analysis had lower levels of IMF than those in the analysis of McGlone *et al.* (2005) or

Andrés *et al.* (2007). Andrés *et al.* (2007) did not apply their models to an independent dataset so the true predictive ability of the model is not known.

Two samples (1 and 154) were excluded form the analysis for moisture content on the basis that they were outliers based on the fact that they were the two extremes of the calibration dataset and because their spectra were also significantly different (Figure 6.8). From Figure 6.8, it can be seen that sample 34 also appears to be an outlier, yet excluding this sample from the analysis did not offer any increase in R^2_{cal} or reduction in SE_{cv} (not shown). This also shows that removing samples based on the Hotelling T^2 ellipse alone is sub-optimal because not all spectra outside the ellipse are outliers. In fact when the Hotelling T^2 ellipse is set with $\alpha = 0.05$, 5% of the samples are expected to fall outside the boundaries of the Hotelling T^2 ellipse.

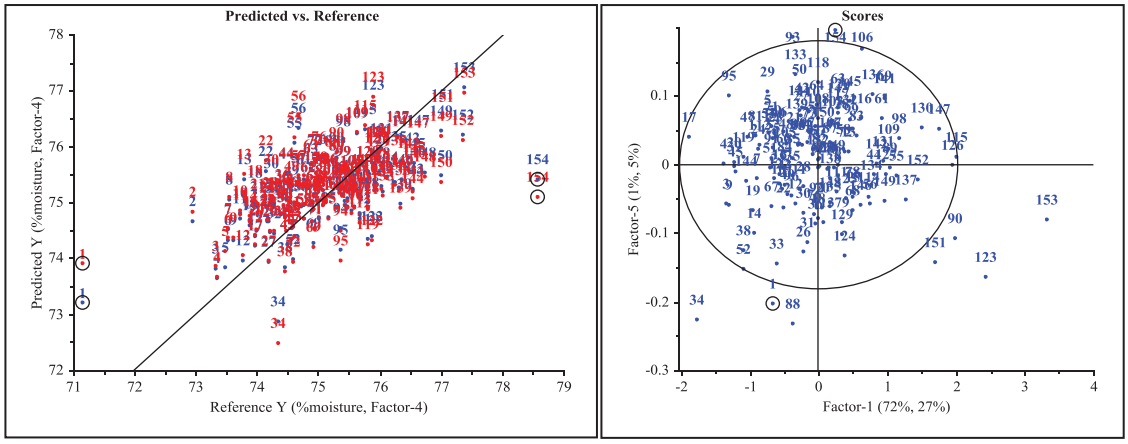

Figure 6.8 Calibration and cross-validation for moisture content (left) and the principal component analysis (right) with a Hotelling T^2 ellipse ($\alpha = 0.05$) superimposed showing that samples numbers 1 and 154 (circled) were deemed to be outliers and were removed from the analysis. The percentage values in brackets indicate the percentage of variation explained in the X variables (the spectra) and the percentage of variation explained in the Y variable (moisture content) respectively.

The R^2_{cal} for measuring the moisture in lamb LL was 38% (SE_{cal} = 0.71%), R^2_{cv} = 32.6% (SE_{cv} = 0.75%, RPD_{cv} = 1.33) and R^2_{pred} = 8.7% (SE_{pred} = 0.91%, RPD_{pred} = 1.06). Cozzolino *et al.* (2000) reported an R^2_{cal} = 56% (SE_{cal} = 12.9 g/kg) and R^2_{cv} = 36% (SE_{cv} = 15.5 g/kg, RPD_{cv} = 1.25) for the moisture content of lamb LL. Andrés *et al.* (2007) reported and R^2_{cv} = 59% with a SE_{cv} = 0.69% (RPD_{cv} = 1.57) which is higher than the R^2_{cv} = 32.6%, SE_{cv} = 0.75% and RPD_{cv} = 1.33 reported in the current analysis.

There was a marked decline in performance for predicting LL pH_{ult} between the calibration, cross validation and prediction phases in the current results, three samples were deemed to be outliers, one value was exceptionally high, (pH_{ult} = 6.34) and the other values were spectral outliers (P = 0.05). After removing three the outliers in the calibration phase, the model failed to account for any of the variation in the prediction set (Table 6.2) which illustrates that cross-validation is not necessarily a good indicator of future predictive ability. The calibration and cross validation performance for LL pH_{ult} (R^2_{cal} = 32% and R^2_{cv} = 13% (SE_{cv} = 0.09, RPD_{cv} = 1.33) was better than the R^2_{cal} of 26% and R^2_{cv} = 7% (SE_{cv} = 0.17, RPD_{cv} = 1.00) reported by Andrés *et al.* (2007). Similar performance for predicting pH_{ult} in beef using NIR has also been observed (RPD_{cv} = 1.12 to 1.26) (Prieto *et al.* 2008).

6.3.2 Prediction of meat quality in leg muscles

Almost all reports of NIR performance for the prediction of meat quality are taken on the LL, because it is a high-value muscle and is relatively easy to recover and test. Much less research effort has focused on characterizing the relationships between muscles within the carcase; so it is not known whether NIR spectra collected on the LL can be used to predict the meat quality characteristics of another muscle. In the current experiment, the ability of NIR spectra from LL to predict a range of instrumental meat quality parameters on the SM and VL was investigated. NIR was poor at predicting the shear force traits of both the SM and VL (Table 6.2) which was expected given that Volodkevitch shear force was found to be poorly correlated between the LL and the VL in these lambs (Lambe *et al.* 2011). A correlation between LL and SM could not be established because two different shear force tests were used. NIR did show some promise for predicting the chroma of lamb SM, R^2_{pred} = 57% (SE_{pred} = 1.03, RPD_{pred} = 1.55), but the performance was not as good for predicting the hue angle (RPD_{pred} = 1.19). RPD_{pred} values of 1.57 for L*, 1.47 for a* and 1.26 for b* were obtained when predicting SM colour from NIR spectra collected on the LL. Interestingly, NIR was able to predict the colour parameters of SM with comparable, if not better accuracy than reports on beef where spectra and meat colour were both recorded on the LL. Andrés *et al.* (2008) reported RPD_{cv} values of 2.07 for L*, 0.90 for a* and 1.37 for b* and Prieto *et al.* (2008) who reported RPD_{cv} values of 1.12 for L*, 1.24 for a* and 0.98 for b* but

in a different experiment, Prieto *et al.* (2009b) reported RPD_{cv} values of 2.47 for L*, 2.02 for a* and 2.48 for b* on 194 beef samples.

There were no previous reports of NIR being used to predict cooking loss in lamb so a comparison on a like-for-like basis was not possible. The calibration, cross-validation and prediction performance for SM cooking loss (RPD_{pred} = 1.21, Table 6.2) was comparable to previous reports on beef; Leroy *et al.* (2004) reported R^2_{cv} = 25% (SE_{cv} = 2.31%, RPD_{cv} = 1.13) on a 189 beef samples, De Marchi *et al.* (2007) reported R^2_{cv} = 10, SE_{cv} = 1.27% (RPD_{cv} = 2.80) for predicting cooking loss on 148 beef samples, Prieto *et al.* (2008) reported an R^2_{cal} of 14% (SE_{cv} = 1.61%, RPD_{cv} = 1.03) on beef *M. longissimus thoracis* from 53 steers and were unable to calibrate models to predict cooking loss on another dataset of 67 young beef animal samples. Furthermore, Cecchinato *et al.* (2011) reported an R^2_{cal} = 4% (SE_{cal} = 3.55%) for the prediction of cooking loss in bull beef but they did not report the standard errors of the cross validation.

6.3.3 Future directions for NIR analysis on lamb

In this experiment, NIR spectra were collected after the lamb was aged for between 7 and 9 days under experimental conditions. In order for NIR to play a role in lamb carcase evaluation, further experimental work is needed to assess whether the models developed here are applicable to un-aged lamb in an abattoir environment. The current analysis was based on a median of 10 replicate spectra per lamb, each taking approximately1-2 seconds to collect. Lamb slaughter plants typically operate at a much higher rate (≈10 lambs per minute) than beef plants, meaning the time budget for NIR scanning is smaller, so collecting replicate spectra per lamb may not be possible. It is not known whether a single NIR scan is sufficient for predicting meat quality, further analysis is needed to determine if a single NIR scan can be used to predict lamb meat quality.

Besides collecting NIR spectra under commercial operating conditions, future experiments should assess NIR performance on a wider range of genotypes typical of the slaughter population. One difference between the lambs in this experiment

compared to the normal slaughter population relates to the IMF%, the levels of IMF% in the Texel breed are low compared to other breeds (Fisher *et al.* 2000; Hopkins *et al.* 2006; Warner *et al.* 2010). In the UK, the average IMF% of *M. longissimus* in lamb chops (mostly from crossbred lambs) purchased at supermarkets was 3.20% (Angood *et al.* 2008).

6.4 Summary and conclusions

1. NIR spectra collected on the LL had a limited ability to predict Volodkevich shear force (RPD_{pred} = 1.17) or MIRINZ shear force (RPD_{pred} = 1.33). Models were unable to correctly identify all samples with shear force values > 5.5 kgF. There are few reports of NIR predicting shear force of lamb in the published literature so a comparison is difficult.

2. Prediction of IMF% was comparable to previous reports R^2_{pred} = 65% (SE_{pred} = 0.33%, RPD_{pred} = 1.68).

3. Prediction of meat moisture content was poor, R^2_{pred} = 8.7% (SE_{pred} = 0.91%, RPD_{pred} = 1.06) which was similar to previous reports on lamb.

4. There are no previous reports where NIR spectra collected on one muscle have been used to predict meat quality parameters of another muscle; but NIR spectra collected on the LL showed some promise to predict colour traits on SM.

5. Shear force traits in SM and VL could not be predicted by NIR spectra collected on the LL.

6. NIR was unable to predict pH_{ult} of LL or SM.

7. These results were obtained on experimental lambs under controlled experimental conditions; future experiments should investigate NIR performance under commercial operating conditions.

7 Meat quality characteristics of the *M. longissimus lumborum* from farmed deer as affected by genotype (red vs. wapiti-red crossbred), sex, sampling location and chilled aging

Presentations based on the results reported in this chapter:

Craigie, C. R., Purchas, R. W., Maltin, C. A., Roehe, R., & Morris, S. T. (2011). The superior tenderness of the posterior part of Longissimus lumborum from farmed deer was no longer evident after aging. *57th International congress of Meat Science and Technology, Ghent, Belgium,* P063.

Abstract

The effects of sex, genotype, chilled aging and location within venison *M. Longissimus lumborum*, (LL) on meat quality were assessed. Venison short-loins recovered from the left side of 12-14 month old pasture-fed deer (n = 79), including red (18 hinds and 20 stags) and wapiti-red crossbreds (20 hinds and 21 stags) 24h post mortem were divided into anterior and posterior halves that were allocated alternately to either a 3d or a 42d aging period at 1 ± 1°C. Meat quality parameters included Warner-Bratzler shear force, ultimate pH (pH_{ult}), purge, water-holding capacity, cooking loss, sarcomere length, and L*a*b* colour parameters. Stags had significantly tougher LL than hinds although the effect was largely due the curvilinear relationship between pH_{ult} and textural traits. Although confounded with farm, and slaughter day effects, genotype had a significant effect on several carcase and meat quality traits with the red deer group having lighter carcases, higher pH_{ult}, lower expressed juice values, shorter sarcomeres, and darker-coloured meat (all $P < 0.01$). Aging affected all meat quality traits except sarcomere length and cooking loss. LL samples from the anterior section of the short-loin had significantly higher shear force ($P < 0.001$), but this difference was not significant after the additional 39d aging.

7.1 Introduction

Tenderness is an important factor contributing to a positive eating quality experience in red meat (Huffman *et al.* 1996). In the case of venison, a low fat content and high iron content add to its perception as a high-value product in many markets (Drew and Seman 1987; Purchas *et al.* 2010). New Zealand exported just over 14.2 million kg of venison in 2010 according to Deer Industry New Zealand (www.dinz.org). The majority of farmed venison produced in New Zealand is destined for EU or USA markets (Hoffman and Wiklund 2006). Vacuum-packaged venison is shipped to the EU in either a chilled or frozen form via sea freight which takes up to 6 weeks, but the effect of this process on product quality, particularly the relationship between shear force measures (which are indicative of tenderness) and ultimate pH (pH_{ult}) is not well characterized.

Approximately 75% of lean meat is made up of water and the retention of water is important for maintaining saleable weight of lean meat and to ensure sufficient juiciness when consumed (Offer and Trinick 1983; Huff-Lonergan and Lonergan 2005). The venison industry (producers and processors) see water loss during chilling, packaging and cooking as especially important because succulence and juiciness (which are influenced by both water and intramuscular fat [IMF]) contribute to a positive eating experience. Cooking methods such as the final internal temperature probably have the largest effect on meat eating quality (Mathoniere *et al.* 2000; Purchas *et al.* 2010), but are beyond the control of the meat value chain. The IMF levels found in venison *M. longissimus lumborum* (LL) are relatively low (< 1-2%) (Kay *et al.* 1981; Stevenson *et al.* 1992; Purchas *et al.* 2010) compared to beef, lamb and pork (Kempster *et al.* 1986) and appear to be lower in *M. semimembranosus* (< 1%) than LL (Stevenson-Barry *et al.* 1999b). The juiciness of *M. semimembranosus* (as assessed by a trained sensory panel) was also found to be lower than LL (Pollard *et al.* 2002) suggesting a link between the two parameters. With such low levels of IMF, minimizing the water loss during processing, storage and cooking of venison will help to optimize juiciness.

Despite the importance of IMF, pH_{ult} arguably plays a greater role in the capacity of cooked venison to retain water and also affects appearance of the vacuum packaged meat product (Wiklund *et al.* 2009). Furthermore, a curvilinear relationship between pH_{ult} and venison LL has been reported where meat with intermediate pH_{ult} (5.8-6.2)

was tougher than LL with normal (5.50 – 5.70) or high pH_{ult} (> 6.20) (Stevenson-Barry *et al.* 1999a).

A rapid drop in muscle temperature before sufficient pH decline *post mortem* can result in an early onset of rigor and "cold shortening" which is linked to toughness in meat (Dransfield and Rhodes 1976). Prime venison carcases may be predisposed to cold shortening due to their low level of subcutaneous fat (which provides insulation against cold ambient temperatures while the muscle pH is still in decline). This phenomenon has been reported in lean veal calves (Williams *et al.* 1987) and lean lambs (Davey and Garnett 1980). As a means of preventing cold shortening, electrical stimulation is applied to all deer slaughtered in New Zealand abattoirs (Wiklund *et al.* 2010b). Besides preventing cold shortening, electrical stimulation is known to accelerate proteolysis and tenderization due to an earlier onset of rigor at higher carcase temperatures in the early post mortem period (Chrystall and Devine 1983; Wiklund *et al.* 2001). In the absence of cold shortening, effects of electrical stimulation on venison quality appear to be minimal where chilled aging exceeds three weeks (Wiklund *et al.* 2001).

Several other processing factors have been shown to influence venison eating quality. Increased chilled aging time (in a vacuum package) reduced venison shear force but the amount of water loss increased with time (Wiklund *et al.* 2001; Farouk *et al.* 2009). The ability of venison LL to retain water was also improved by chilled aging for 1-2 weeks at –1.5°C prior to freezing for eight weeks (Farouk *et al.* 2009). Consumers determined that the venison from this treatment had a higher overall liking than alternative treatments (no aging, aged for three weeks and frozen for six weeks) but was less tender and was as juicy as samples that were chilled for nine weeks and never frozen (Farouk *et al.* 2009). Interestingly, the shear force of venison *M. biceps femoris* with intermediate pH_{ult} aged for up to 42d and frozen was significantly more tender than the chilled aged (never frozen) product (Stevenson-Barry *et al.* 1999a). Other processing factors such as the method of carcase suspension (pelvic or Achilles tendon) during conditioning also affects consumer perceptions of venison juiciness, tenderness and overall liking, with significant improvements attained in all three parameters in *M. gluteus medius* using the pelvic method (Hutchison *et al.* 2010). The application of a fine water mist during chilling (known as spray chilling) appeared to reduce the water loss of venison flap and shoulder meat but had no effect on the moisture content or

eating quality parameters of LL in the study of Wiklund *et al.* (2010b). Besides processing effects, production factors such as sex, slaughter age, stress and diet have all been found to affect venison quality parameters. As has been seen in other species, LL from males (stags) has been reported to be significantly tougher with lower levels of IMF than that from females (hinds) (Purchas *et al.* 2010). One report has also shown that shear force of stag venison increases with increasing age at slaughter (Stevenson-Barry *et al.* 1999b). A comparison of paddock-shot and deer-slaughter-plant handling/slaughter procedures showed that stress from handling had minor effects on meat quality (Pollard *et al.* 2002) although the number of deer in the experiment was small. Finishing diet can also affect venison quality. Grass-based pasture or pellet-based feed have been shown to affect venison fatty acid profile but diet was reported to have little impact on venison sensory parameters (Wiklund *et al.* 2003).

Genotype (such as red vs. wapiti-red crossbreds) affects growth rates in farmed deer (Hoskin *et al.* 1999) and may also affect venison quality parameters. A general lack of evidence surrounds possible effects of the wapiti-red crossbred genotype on venison eating quality. Similarly, there is little information regarding the effects of intramuscular sampling location on venison eating quality parameters, despite muscle location effects having been reported in other species (Hansen *et al.* 2004; Shackelford *et al.* 2004; Wheeler *et al.* 2007).

There are relatively few reports on venison meat quality from farmed red or wapiti-red crossbred deer and previous studies have typically involved relatively low numbers of animals (≤ 20 per group) (Stevenson *et al.* 1992; Stevenson-Barry *et al.* 1999a; Stevenson-Barry *et al.* 1999b; Wiklund *et al.* 2001; Wiklund *et al.* 2003; Farouk *et al.* 2009; Purchas *et al.* 2010) and may not always be representative of the typical age (12-14 months old) or genotype profile of farmed deer currently produced and processed in New Zealand (Asher *et al.* 2011).

The aim of this experiment was to:

- Investigate the effects of sex, genotype, sampling location and chilled aging on meat quality parameters of venison short-loin produced, processed, and aged under commercial conditions.

7.2 Materials and methods

7.2.1 Animals

The 79 deer (*Cervus elaphus*) for the experiment were processed under commercial conditions in two batches. The first batch was slaughtered on Monday the 14th of December 2009 and consisted of 18 red hinds and 20 red stags, and the second batch was slaughtered on Wednesday the 16th of December 2009 and consisted of 20 wapiti-red crossbred hinds and 21 wapiti-red crossbred stags. The two batches came from two different farms, but deer within each batch were run together up to slaughter.

7.2.2 Abattoir protocol

Deer were immobilized by a captive bolt pistol. Low voltage electrical stimulation was applied (72 volts for 62 seconds with 7.5 millisecond stimulations at intervals of 70 milliseconds). Carcases were exsanguinated immediately after stunning and dressed according to normal commercial practice. The dressing and subsequent weighing (hot carcase weight, HCW), grading (soft tissue depth over the 12th rib vertically down from the hip bone (*tuber coxae*) and inspection process before entry to the chiller took approximately 16 minutes. Carcases were chilled overnight at 1 ± 1°C. Cold carcase weight was recorded as carcases entered the boning room at approximately 24 hours *post mortem*. *M. Longissimus lumborum* (LL) short-loin samples from the left side of the carcase were recovered from between the last rib and the pelvic bone. Each short-loin sample was halved and the two halves were weighed, vacuum-packaged and the anterior and posterior halves were allocated alternatively to a 3 day (3d) or 42 day (42d) aging period. Samples of short-loin were aged at 1 ± 1°C under commercial conditions for the designated aging time prior to freezing at –30°C for at least 1 week.

7.2.3 Meat quality assessment

LL samples were defrosted in batches of 8 at 1 ± 1°C for 22 hours, removed from vacuum packs, and blotted dry using paper towels before the thawed weight was recorded. A total of 78 samples were included in the meat quality analysis due to the misallocation of two half short-loins to 3d day and 42d aging treatments which resulted

in a missing data point at each aging time. Purge was calculated by subtracting the weight of the LL sample upon opening from the weight at packing and expressing the difference as a percentage of the packing weight. A 25 mm steak removed from the middle of the short-loin was cooked for 90 minutes at 70°C within plastic bags that were suspended in a water bath (Purchas and Aungsupakorn 1993). Following cooking, samples were stored overnight at 1 ± 1°C, and then five 13 x 13 mm-cross-sectioned cores were prepared in such a way that muscle fibres ran longitudinally in the core. Measurements were made with a Warner-Bratzler device (crosshead speed of 230 $mm \bullet min^{-1}$; G-R Electric Mfg. Co., Manhattan, KS) fitted with a square blade and a 30-kg load cell (Purchas and Aungsupakorn 1993). Two shears perpendicular to the muscle fibre axis were made per core. Parameters recorded for each shear were the average initial yield force (IYF), the peak shear force (WBSF), and the average shear force through the duration of the shear as an index of the work done (WD) (Purchas and Aungsupakorn 1993). The difference between the peak shear force and the initial yield has been linked to the connective tissue component of meat toughness (Beilken *et al.* 1986) so this difference was also calculated (WBSF – IYF). For each shear force trait, the average of the 10 replicate shears was taken as the final reading.

The remaining anterior section of the short-loin sample was used for measuring sarcomere length, pH_{ult} and colour. The anterior portion was halved laterally; one half was frozen at –30°C for subsequent colour analysis and the other half retained on ice for pH and sarcomere length measures. The sections for colour analyses, which included the half where the epimysium was thinnest, were defrosted for 12 hours at 1 ± 1°C, then cut across the fibres and allowed to bloom for 20 minutes at room temperature. Preliminary trials indicated that 20 minutes was sufficient bloom time for venison. Two measures of L*, a* and b* were made using a Minolta Chroma meter (CR-200, 8 mm measured area diameter, standard illuminant C, calibrated with a CM-101W white tile; Ramsey, NJ) and the average calculated. Ultimate pH (pH_{ult}) was assessed on a homogenate prepared from 2.0 to 2.5 g of meat in 10 mL of distilled water using a combination pH electrode (Jenway 3020 pH meter with automatic temperature compensation). Sarcomere length (SL) was assessed by laser diffraction (Boulton *et al.* 1973). The average distance between 1^{st} order diffraction patterns was calculated for 12 patterns (Cross *et al.* 1981).

Expressed juice (EJ) as an indicator of water-holding capacity was evaluated using a filter paper press method based on that described by (Hamm 1986). A 500 ± 10 mg sample of thawed LL was removed from the centre of the short-loin sample, placed on a sheet of Whatman No. 1 filter paper. The paper and sample were placed between two perspex plates and a force of 10 kg applied for 5 minutes. After removal from the plates, the outline of the meat was marked on the underside of the filter paper. Samples were set aside to dry before the area of the juice-stained region was measured using a digital planimeter (Placom KP-90N). Expressed juice (EJ) was calculated by dividing the area by the sample weight yielding a value in cm^2g^{-1}. Three samples were excluded from the analysis because damp filter paper accentuated the juice staining.

7.2.4 Statistical analysis

Descriptive statistics (Table 7.1) were produced using the MEANS procedure of SAS (SAS Inst. Inc., Cary, NC). Variance components were estimated with the restricted maximum likelihood (REML) method using the MIXED procedure of SAS. Models for carcase traits included sex (hinds, n = 38 and stags, n = 41) and genotype (wapiti-red crossbreds, n = 38 and reds, n = 41), short-loin weight and chill loss were adjusted for HCW (Table 7.2). Least-squares (L-S) means for each sex and genotype level and the standard error of the difference (SED) between the means in each group were generated using the L-S means option in SAS. For analysing the meat quality traits, aging time (3 or 42d) and sampling location (anterior and posterior) were combined to form a new class variable with four levels (anterior 3d, posterior 3d, anterior 42d and posterior 42d). Along with sex and genotype, the new class variable was included as a fixed effect, and animal was fitted as a random effect in the mixed model. The effects of aging time (3 or 42d) and sampling location (anterior and posterior) were calculated using two estimate statements in SAS. The first estimate statement was used to test the aging effect on meat quality traits and gives the difference between the two aging times (3d value – 42d value). The second estimate statement was used to determine the sampling location effect on meat quality and gives the difference between sampling locations (posterior value – anterior value). For all meat quality traits, L-S means, are presented with and without adjustment for pH_{ult} as linear and quadratic covariates. The REG procedure of SAS was used to characterise the curvilinear relationship and 95% confidence intervals between pH_{ult} and WBSF (Figure 7.1). Multiple pair-wise comparisons were made for

peak shear force at each age-location combination after Bonferroni adjustment (Figure 7.2). HCW was tested as a covariate for meat quality traits but was not statistically significant, and no statistically significant interactions between sex and genotype were found for any traits.

7.3 Results and discussion

Descriptive statistics including the number of observations for each trait, mean, standard deviation, coefficient of variation (CV), and range are shown in Table 7.1.

7.3.1 Effects of sex on carcase traits

The effects of sex on carcase traits are presented in Table 7.2; there was no significant difference in HCW between hinds and stags, possibly because heavier stags had been slaughtered in an earlier draft. Stags are usually heavier than hinds when finished on pasture (Hoskin *et al.* 1999; Purchas *et al.* 2010). There were no significant sex effects on the soft tissue depth (GR) or the weight or yield (2.24% of HCW for both sexes) of the short-loin muscle. The chill loss (%) was highly variable CV of 54.12% (Table 7.1). Chill loss was significantly greater in stags than hinds when adjusted for HCW effects (P = 0.007). There are few reports detailing the effect of sex on chill loss on deer carcases so comparison is difficult. In a recent experiment involving Iberian x Duroc pigs, no differences in chill loss were found between intact females, castrated females or castrated males (Serrano *et al.* 2009).

There was no significant effect of pH_{ult} recorded in the short-loin on carcase chill loss (data not shown). GR also had a significant effect on chill loss when included as a second covariate (0.11 percentage point increase in chill loss per millimetre increase in GR, P = 0.03) but estimations of sex effects were very similar (data not shown). Hinds tend to be fatter than stags at a the same carcase weight (Stevenson-Barry *et al.* 1999b) but there was no difference between the sexes in the current analysis, a lower fat cover may have explained the greater chill loss observed in stags.

Table 7.1 Descriptive statistics for carcase and meat quality traits at both 3d and 42d aging times for the 79 deer (38 hinds and 41 stags) used to assess the effects of sex and genotype, aging time and sampling location.

Trait (abbreviation)	Aging	*n*	Mean	SD	CV (%)	Range
Hot carcase weight (HCW) (kg)	-	79	54.39	5.01	9.20	45.90-74.70
Short-loin weight (g)	-	79	609.90	93.24	15.29	330.00-824.00
Soft tissue depth (GR) (mm)	-	79	4.56	1.71	37.48	2.00-8.00
Chill loss (%)	-	79	0.98	0.53	54.12	0.00-3.23
Ultimate pH (pH_{ult})	3d	78	5.59	0.17	3.09	5.42-6.31
	42d	78	5.61	0.16	2.93	5.41-6.25
Purge (%)	3d	78	2.96	1.05	35.30	0.00-5.53
	42d	78	4.93	1.50	30.36	2.00-8.85
Expressed juice (EJ) (cm^2g^{-1})	3d	75	31.63	2.98	9.43	22.48-39.10
	42d	78	27.09	2.81	10.36	21.22-32.87
Cooking loss (CL) (%)	3d	78	28.28	2.32	8.21	21.76-32.19
	42d	78	27.87	1.59	5.72	22.55-30.96
Sarcomere length (SL) (µm)	3d	78	1.58	0.10	6.43	1.23-2.00
	42d	78	1.56	0.08	5.15	1.23-1.71
Lightness (L*)	3d	78	35.31	2.37	6.72	29.66-41.12
	42d	78	37.41	1.79	4.77	30.73-40.09
Redness (a*)	3d	78	11.71	1.60	13.66	6.87-14.86
	42d	78	12.34	1.38	11.18	7.70-14.81
Yellowness (b*)	3d	78	2.95	0.84	28.65	0.92-4.75
	42d	78	3.28	0.71	21.50	1.39-4.58
Warner-Bratzler shear force (WBSF) (kgF)	3d	78	7.64	1.81	23.68	3.90-13.07
	42d	78	5.12	1.26	24.63	3.13-9.52
Initial yield force (IYF) (kgF)	3d	78	6.71	1.60	23.83	3.26-11.57
	42d	78	4.29	1.04	24.23	2.64-7.74
WBSF – IYF (kg)	3d	78	0.93	0.49	53.20	0.15-2.58
	42d	78	0.83	0.48	57.78	0.15-2.36
Work done (WD)	3d	78	2.30	0.51	22.22	1.06-3.48
	42d	78	1.66	0.38	23.12	1.00-2.93

7.3.2 Effects of genotype on carcase traits

Effects of genotype on carcase traits are shown in Table 7.2. Despite the fact that genotype (red or wapiti-red crossbred) was confounded with farm and slaughter day effects, both farmers were members of a high-welfare producer group and followed a very similar finishing model. The processing protocol was the same on both days. Both farmers finished stags and hinds of similar ages together on pasture up to the day of slaughter. In light of this, it is assumed that differences between the animals are due to genotype rather than environmental factors. Wapiti-red crossbred deer had heavier carcase weights, greater soft tissue depths, greater short-loin weights ($P < 0.001$). By adjusting the short-loin weight for HCW, it can be seen that short-loin was 54 g heavier in the crossbred deer. In contrast to sex, genotype had no significant effect on chill loss, although chill loss tended to be higher in red deer ($P = 0.08$).

Table 7.2 Least-squares means showing the effects of sex (hinds and stags) and genotype (wapiti-red crossbred and red) on deer carcase weight, short-loin weight, soft tissue depth and chill loss.

	Sex				Genotype				
Trait[a]	Stags	hinds	SED[b]	Effect	Crossbred	Red	SED[b]	Effect	HCW[c]
	n = 41	*n* = 38			*n* = 38	*n* = 41			
HCW (kg)	53.8	55.2	0.98	0.16	57.0	52.0	0.98	< 0.001	-
Short-loin weight (g)	611	610	13	0.98	638	584	15	< 0.001	< 0.001
GR (mm)	4.59	4.62	0.26	0.92	5.87	3.34	0.26	< 0.001	ns
Chill loss (%)	1.13	0.81	0.11	0.007	0.85	1.08	0.13	0.08	0.016

[a] HCW = Hot carcase weight, the following traits were adjusted for HCW: Short-loin weight, GR = Soft tissue depth, Chill loss = percentage difference between hot and cold carcase weight.
[b] SED = Standard error of the difference between predicted means.
[c] Effect (*P* value) of HCW (included as a covariate) on venison carcase quality traits, ns = not significant and not included.

7.3.3 Effects of HCW on carcase traits

For every kilogram increase in HCW, there was an 11 g increase in short-loin weight ($P < 0.001$); similarly there was a 0.03 percentage point increase in chill loss per kg (data not shown). GR was not significantly affected by HCW in the current analysis, probably because the relationship between GR and HCW in stags appears at higher carcase weights (linear trend) and in hinds where carcases are excessively fat (exponential trend) (Stevenson-Barry *et al.* 1999b).

7.3.4 Effects of sex and genotype on meat quality traits

7.3.4.1 Ultimate pH

There were no significant differences in pH_{ult} between hinds and stags, but reds had significantly higher pH_{ult} values than crossbreds (Table 7.3). A curvilinear relationship between ultimate pH and shear force has previously been suggested for venison in an experiment with a small number of deer ($n = 18$) and no sex effects were accounted for in that analysis (Stevenson-Barry *et al.* 1999a). In light of this, pH_{ult} was tested simultaneously as both a linear and quadratic covariate in models for meat quality traits in order to determine whether the sex and, in particular, the genotype effects were due to differences in LL pH_{ult} (Table 7.3). There was evidence in the current results to support the previous findings of Stevenson-Barry *et al.* (1999a) who reported a curvilinear relationship between peak shear force and pH_{ult} (Table 7.5). Furthermore, there was evidence of a curvilinear relationship between pH_{ult} and cooking loss (Table 7.3), IYF, WBSF – IYF and WD (Table 7.5). A curvilinear relationship between pH_{ult} and Warner-Bratzler peak shear force has been reported in beef (Purchas 1990; Purchas and Aungsupakorn 1993; Jeleníková *et al.* 2008) and lamb (Devine *et al.* 1993). In order to further investigate this observation, 3d and 42d shear force values were plotted against the averaged pH_{ult} from the two aging times (3 and 42d) as well as the fitted value, and 95% confidence intervals for the average WBSF of 3d and 42d values (Figure 7.1). A curvilinear relationship was observed and characterized by the following equations: WBSF 3d (kg) = $-15.868\ (pH_{ult}^2) + 188.87\ (pH_{ult}) - 551.94$ ($R^2 = 30\%$, RSD = 1.53), WBSF 42d (kg) = $-12.896\ (pH_{ult}^2) + 154.38\ (pH_{ult}) - 451.77$ ($R^2 = 39\%$, RSD = 1.00). By analysing the relationship between shear force determined using MIRINZ

tenderometer (Macfarlane and Marer 1966) and pH_{ult} data from abattoir records of deer of unknown background slaughtered at the same plant between 2000 and 2009 (*n* = 120), a similar curvilinear relationship was observed: MIRINZ PF (kg) = – 15.397(pH_{ult}^2) + 177.35(pH_{ult}) – 506.39 (R^2 = 20%, RSD = 1.28) (data not shown).

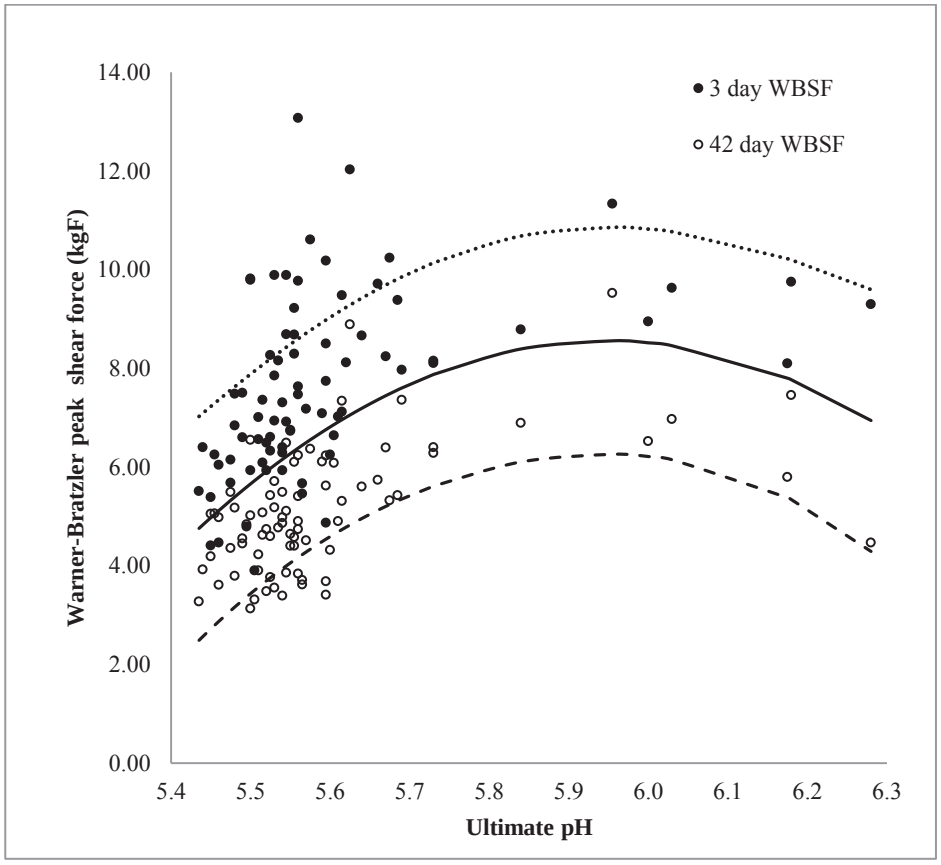

Figure 7.1 The curve for the quadratic regression equation (solid line) for the relationship between ultimate pH and the average of the 3d and 42d Warner-Bratzler peak shear force (WBSF) values for venison *M. longissimus lumborum*, together with the 95% confidence intervals (dotted and dashed lines). Individual data points for the 3d and 42d WBSF values are shown. Equation: Average WBSF (kgF) = –14.525(pH_{ult}^2) + 172.74 pH_{ult} – 505.05, (R^2 = 40%, RSD = 1.10). Separate equations for the 3d and 42d data are given in the text.

Table 7.3 Least-squares means showing the effects of sex (hinds and stags), genotype (wapiti-red crossbred and red), aging (3 – 42d value) and sampling location (posterior – anterior value) on venison short-loin quality traits as well as the linear and quadratic effects of ultimate pH.

Trait[a]	Sex				Genotype				Covariate[d]	Other effects[e]			
	stags	hinds	SED[b]	*P*[c]	Crossbred	Red	SED[b]	*P*[c]	pH_{ult}, pH_{ult}^2	Age	*P*	Loc	*P*
pH_{ult}	5.62	5.57	0.04	0.18	5.53	5.65	0.03	0.001	-, -	–0.02	< 0.001	–0.01	0.03
Purge (%)	3.77	4.16	0.21	0.07	4.20	3.74	0.21	0.03	-, -	–1.93	< 0.001	0.70	< 0.001
EJ (cm^2g^{-1})	29.83	29.06	0.46	0.10	30.72	28.17	0.46	< 0.001	-, -	4.64	< 0.001	0.26	0.50
CL (%)	28.48	27.63	0.39	0.03	27.99	28.12	0.38	0.74	-, -	0.38	0.10	–0.36	0.12
SL (µm)	1.56	1.59	0.02	0.08	1.60	1.54	0.02	0.001	-, -	0.02	0.02	0.00	0.61
Adjusted for pH_{ult}													
Purge (%)	3.80	4.12	0.21	0.13	4.11	3.81	0.22	0.19	0.46, 0.42	1.95	< 0.001	0.68	< 0.001
EJ (cm^2g^{-1})	29.88	28.96	0.45	0.05	30.47	28.37	0.46	< 0.001	0.30, 0.27	4.61	< 0.001	0.19	0.61
CL (%)	28.55	27.52	0.31	0.001	27.66	28.42	0.32	0.02	0.01, 0.01	0.36	0.13	–0.39	0.10
SL (µm)	1.56	1.58	0.01	0.39	1.58	1.56	0.01	0.17	0.09, 0.12	0.01	0.18	–0.01	0.24

[a] pH_{ult} = ultimate pH, Purge = fluid exuded in vacuum package after freezing and thawing, EJ = expressed juice, CL = cooking loss, SL = sarcomere length.
[b] SED = Standard error of the difference between predicted means.
[c] Effect (*P* value) of sex and genotype on venison loin meat quality.
[d] Effect (*P* value) of pH_{ult} included as linear and quadratic covariates.
[e] Other effects include the differences between aging time (Age; 3d – 42d value) and sampling location (Loc; posterior – anterior value) as well as the significance (*P* values) of the effects on venison short-loin meat quality.

7.3.4.2 Purge

Purge was highly variable in the current study with coefficients of variation > 30% at both aging periods (Table 7.1). The percentage of purge in stag short-loin was 3.77% (Table 7.3) which is similar to the ~4% purge at 2d aging and the 3.5% purge at 42d reported for eight stags by Farouk *et al.* (2009). The amount of purge tended to be higher in LL from stags (P = 0.07) but there was no significant difference after adjustment for pH_{ult} (P = 0.13). LL from the crossbred genotype had a greater amount of purge than reds (P = 0.03) but the difference was no longer statistically significant after adjusting for pH_{ult} (P = 0.19) (Table 7.3). This may be because the higher pH_{ult} for reds allowed more calpastatin activity which inhibits the calpain enzymes responsible for proteolysis thus resulting in less proteolysis and less purge (Wiklund *et al.* 2010a).

7.3.4.3 Expressed juice (water holding capacity)

There was no significant difference in EJ between the sexes, but after adjustment for pH_{ult}, LL from hinds had a greater propensity to retain water than LL from stags (P = 0.05). This finding is in contrast to the results of Farouk *et al.* (2009) who found no effect of pH on water holding capacity in venison short-loin using the Honikel bag method (Honikel 1998). The current results are consistent with the findings of Purchas *et al.* (2010) who reported no differences in EJ between stags and hinds after 7d aging, although the effect of pH_{ult} on EJ was not considered in that experiment. In the present experiment, venison short-loin from reds had significantly less EJ than crossbreds both before and after adjustment for pH_{ult} (P < 0.001) (Table 7.3). The genotype effect on EJ remained significant after inclusion of pH_{ult} covariates, so appears to be independent of pH_{ult} in this case.

7.3.4.4 Cooking loss

Short-loin from stags had a greater cooking loss than hinds (P = 0.03) (Table 7.3). The magnitude and significance of the sex effect increased after adjustment for pH_{ult}. No statistically significant genotype effects on cooking loss were initially observed, but after inclusion of pH_{ult}, LL from reds had significantly higher cooking loss than crossbreds (P = 0.02).

7.3.4.5 Sarcomere length

Sarcomere lengths were similar to values reported by Purchas *et al.* (2010) on short-loin recovered from the same abattoir using the same laser diffraction methodology, but less than values reported by Wiklund *et al.* (2001) who used the phase electron microscopy method. In the present study, no statistically significant differences in sarcomere length were noted between the sexes, although stags tended to have shorter sarcomeres than hinds ($P = 0.08$). In terms of the genotype effect, crossbreds had longer sarcomeres than reds ($P < 0.001$). Inclusion of pH_{ult} nullified the genotype effect on sarcomere length (Table 7.3) which suggests the LL from red deer may have entered rigor at a higher temperature, although the relationship between sarcomere length and rigor temperature is not well understood (Bekhit *et al.* 2007).

7.3.4.6 Meat colour

LL from stags was significantly more yellow than LL from hinds ($P = 0.03$) (Table 7.4) although this difference was no longer statistically significant after adjusting for pH_{ult} ($P = 0.14$). No differences in colour were observed between the sexes for lightness or redness (Table 7.4). LL from crossbreds was significantly lighter ($P = 0.005$) and more yellow ($P = 0.04$) than LL from reds but the differences in LL colour were no longer statistically significant after adjusting for pH_{ult} (Table 7.4).

7.3.4.7 Shear force

A similar level of variation was present for all shear force traits (CV ranging from 22-24%, Table 7.1). As expected, WBSF – IYF had approximately twice as much variation (CV = 55%), due to the fact that it is derived from the peak shear force and IYF. Variability in WBSF – IYF reflects the variability in the shape of the force deformation curve.

Table 7.4 Least-squares means showing the effects of sex (hinds and stags), genotype (wapiti-red crossbred and red), aging (3 – 42d value) and sampling location (posterior – anterior value) on venison short-loin colour traits as well as the linear and quadratic effects of ultimate pH.

Trait[a]	Sex				Genotype				Covariate[d]	Other Effects[e]			
	stags	Hinds	SED[b]	*P*[c]	Crossbred	Red	SED[b]	*P*[c]	pH_{ult}, pH_{ult}^2	Age	*P*	Loc	*P*
L*	36.20	36.60	0.36	0.26	36.91	35.89	0.35	0.005	-, -	–2.06	< 0.001	0.57	0.05
a*	11.88	12.20	0.29	0.28	12.30	11.78	0.29	0.07	-, -	–0.59	< 0.001	0.50	0.002
b*	2.96	3.30	0.15	0.03	3.29	2.97	0.15	0.04	-, -	–0.31	< 0.001	0.33	< 0.001
Adjusted for pH_{ult}													
L*	36.31	36.45	0.31	0.67	36.59	36.17	0.33	0.21	0.74, 0.83	–2.18	< 0.001	0.44	0.15
a*	12.03	12.02	0.21	0.07	11.93	12.12	0.22	0.40	0.43, 0.56	–0.73	< 0.001	0.39	0.01
b*	3.04	3.19	0.10	0.14	3.08	3.15	0.11	0.50	0.13, 0.19	–0.39	< 0.001	0.27	< 0.001

[a] L* = Lightness, a* = Redness, b* = Yellowness, other superscripts as per Table 7.3 (above).

Table 7.5 Least-squares means showing the effects of sex (hinds and stags), genotype (wapiti-red crossbred and red), aging (3 – 42d value) and sampling location (posterior – anterior value) on venison short-loin shear force traits as well as the linear and quadratic effects of ultimate pH.

Trait[a]	Sex				Genotype				Covariate[d]	Other Effects[e]			
	Stags	hinds	SED[b]	*P*[c]	Crossbred	Red	SED[b]	*P*[c]	pH_{ult}, pH_{ult}^2	Age	*P*	Loc	*P*
WBSF (kgF)	6.74	5.95	0.30	0.01	6.32	6.37	0.30	0.86	-, -	2.46	< 0.001	–0.59	< 0.001
IYF (kgF)	5.80	5.16	0.26	0.01	5.57	5.38	0.25	0.46	-, -	2.37	< 0.001	–0.54	< 0.001
WBSF – IYF (kgF)	0.94	0.80	0.09	0.14	0.75	0.99	0.09	0.01	-, -	0.09	0.07	–0.06	0.28
WD (kgF)	2.11	1.83	0.09	0.002	1.97	1.97	0.08	0.93	-, -	0.62	< 0.001	–0.17	< 0.001
Adjusted for pH_{ult}													
WBSF (kgF)	6.60	6.13	0.25	0.06	6.61	6.12	0.26	0.06	0.004, 0.006	2.64	< 0.001	–0.48	0.002
IYF (kgF)	5.71	5.27	0.23	0.06	5.74	5.24	0.24	0.04	0.01, 0.01	2.49	< 0.001	–0.46	0.002
WBSF – IYF (kgF)	0.89	0.87	0.06	0.79	0.88	0.87	0.06	0.88	0.03, 0.05	0.15	0.007	–0.02	0.72
WD	2.07	1.89	0.07	0.01	2.05	1.91	0.07	0.06	< 0.001, < 0.001	0.68	< 0.001	–0.13	0.004

[a] WBSF = Warner-Bratzler peak shear force, IYF = Initial yield force, WBSF – IYF = connective tissue, WD = Work done.
[b] SED = Standard error of the difference between predicted means [c] Effect (*P* value) of sex and genotype on venison loin meat quality.
[d] Effect (*P* value) of pH_{ult} included as linear and quadratic covariates.
[e] The differences between aging time (Age; 3 – 42d value) and sampling location (Loc; posterior – anterior value) as well as the significance (*P* values) of the effects on venison short-loin meat quality.

Significant sex effects on LL were present for WBSF (P = 0.01), IYF (P = 0.01) and WD (P = 0.002) (Table 7.5). Purchas *et al.* (2010) reported significantly higher shear force values in LL from stags (8.94 ± 0.20 kg) than hinds (6.20 ± 0.57 kg) after 7d aging, although no adjustment was made for pH_{ult}. After adjustment for pH_{ult} in the present analysis (which was statistically significant for all traits); the sex effect was less pronounced for all traits with stag LL tending to have a higher WBSF and IYF than hind LL (P = 0.06), WD remained significant (P = 0.01) but WBSF – IYF was no longer significantly different between the sexes (P = 0.79) (Table 7.4). The meat from males of several species has been found to be tougher than females (or castrates), for example; sheep (Johnson *et al.* 2005), pigs (Gullett *et al.* 1993), goats (Johnson *et al.* 1995) and cattle (Purchas *et al.* 2002a). In terms of the genotype effects on shear force traits, WBSF – IYF was significantly higher in LL from reds (P = 0.01) (Table 7.5). After adjustment for pH_{ult}, WBSF and WD tended to be higher in crossbreds (P = 0.06) while the effect on WBSF – IYF was no longer present, although LL from crossbreds did have a higher IYF than reds (P = 0.04).

7.3.5 Effect of aging on venison quality

Aging venison short-loin for an additional 39d had significant effects on most measured traits (Table 7.3, Table 7.4 and Table 7.5). LL pH_{ult} increased marginally with aging (P < 0.001), purge increased and expressed juice decreased with aging (P < 0.001). Sarcomere length increased slightly with aging, although the effect was no longer significant after adjustment for pH_{ult}. Cooking loss in venison LL was unaffected by aging time (Table 7.3). Aging effects on LL colour were present both before and after adjusting for pH_{ult}; LL became lighter, redder and more yellow after the additional 39d aging (Table 7.4). As expected, the shear force of venison LL decreased with aging (Table 7.5). WBSF, IYF and WD were all significantly lower after the additional 39d aging; (P < 0.001) and WBSF – IYF tended to decrease (P = 0.07). After adjustment for ultimate pH, all shear force traits were significantly reduced after the additional aging (P < 0.01) (Table 7.5).

7.3.6 Effects of sampling location (posterior and anterior) on short-loin quality

The sampling location within the LL had significant effects on most meat quality traits (Table 7.3, Table 7.4 and Table 7.5). The pH_{ult} was significantly lower ($P = 0.03$) and purge significantly higher ($P < 0.001$) in the posterior section of LL, although the location effect on purge remained significant ($P < 0.001$) after adjustment for pH_{ult} (Table 7.3). There were no significant location effects on EJ, CL or sarcomere length (Table 7.3).

The posterior section of LL was significantly lighter ($P = 0.05$), more red ($P = 0.002$) and more yellow ($P < 0.001$) than the anterior section, although the differences in lightness were no longer statistically significant after adjustment for pH_{ult} (Table 7.3). In terms of the location effect on shear force traits, WBSF, IYF and WD were significantly higher at the anterior end of LL ($P < 0.001$) and WBSF – IYF did not differ significantly between locations (Table 7.5). Inclusion of pH_{ult} had little effect on shear force; although the difference was smaller suggesting the differences in shear force can be partly attributed to differences in pH_{ult} along the muscle (Table 7.5). There was an interaction of sampling location and aging time for shear force traits, indicating that the aging effect on peak shear force was greater in the anterior section of the short-loin after accounting for sex, genotype and pH_{ult} effects (Figure 7.2), so that the location effect was no longer significant after 42d aging.

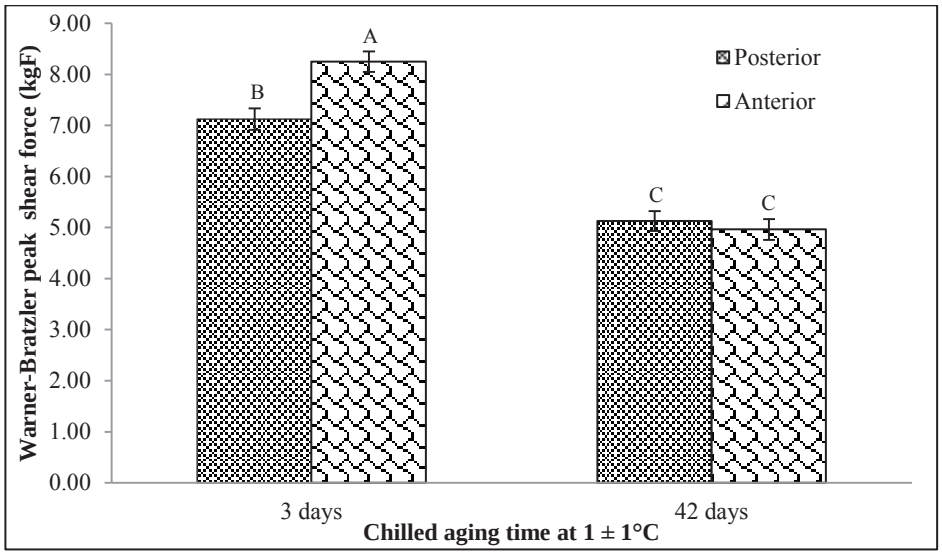

Figure 7.2 Least-squares means and standard error bars for Warner-Bratzler peak shear force of venison *M. longissimus lumborum* showing the aging effect after adjustment for sex, genotype and ultimate pH as both a linear and quadratic covariate. Bars sharing a common letter are not statistically different from each other ($P > 0.05$).

Longitudinal variation in *M. Longissimus thoracis et lumborum* shear force has been previously reported in pork (Hansen *et al.* 2004), beef (Wheeler *et al.* 2007) and lamb (Shackelford *et al.* 2004). In pork, the shear force increased towards the posterior end of the muscle. In beef, the anterior and posterior ends of the muscle were significantly tougher than the middle portion, and in lamb; the muscle was significantly tougher in the anterior section after 14d aging. Intramuscular shear force variation has also been identified in beef *M. adductor femoris, M. biceps femoris, M. semimembranosus and M. semitendinosus* (Senaratne *et al.* 2011).

7.3.7 Future directions for venison meat quality research

As was mentioned previously, genotype was confounded with farm and slaughter day effects in the analysis, further research is needed to verify the apparent gender effects on venison meat quality by finishing both genotypes together on the same farm and slaughtering them on the same day. Further research is needed to establish whether current results are applicable to other muscles in the venison carcase besides the LL. More research is needed to determine the relationship between instrumental measures of venison quality and sensory evaluation of venison eating quality to aid interpretation of results with regard to the likely implications of production and processing decisions.

7.4 Summary and conclusions

1. Stag carcases had a higher chill loss than hinds, and LL from stags had less vacuum packaging purge, lower water holding capacity and a higher cooking loss than LL from hinds.

2. Stags LL had a higher shear force than hinds, although the difference was partly attributable to the higher pH_{ult} in LL from stags.

3. Crossbred deer had higher carcase weights, higher yields of short-loin and a greater soft tissue depth (GR) than red deer, and they also tended to suffer less chill-loss than red deer.

4. LL from red deer had a significantly higher pH_{ult} than crossbreds, after adjusting for pH_{ult} effects; LL from crossbreds had a lower ability to retain water under pressure and a lower cooking loss than LL from reds.

5. After adjusting shear force traits for pH_{ult}, LL from crossbreds tended to have higher shear force values than LL from reds.

6. Several quality LL venison quality traits were affected by aging and sampling location: The additional 39d aging resulted in an increased level of vacuum package purge and an increase in the ability of LL to retain water. The extra aging also increased the lightness, redness and yellowness of LL.

7. As expected, the additional aging resulted in a reduction in all shear force parameters.

8. The anterior section of the short-loin had a higher pH_{ult} and a lower purge than the posterior section.

9. The posterior was more red and yellow than the anterior and had a lower shear force values than the anterior section.

10. In terms of shear force traits, the anterior section of LL also showed a greater response to the additional 39d of aging.

11. The relationship between shear force and pH_{ult} was found to be curvilinear with a maximum peak force at about pH_{ult} of 6.0.

8 Investigations into relationships between visible-near infrared (NIR) spectra and instrumental meat quality parameters in aged and un-aged venison *M. longissimus lumborum*

Abstract

Visible-near infrared (NIR) spectroscopy has shown promise for predicting meat quality in beef and lamb, but it is not known whether it can be used to predict venison meat quality. A non-destructive method for determining venison meat quality parameters would enable venison processors to identify and isolate meat of inferior quality which could improve the average quality of product delivered to specific markets. The aim of this experiment was to determine whether NIR spectra can be used to predict instrumental meat quality parameters of venison short-loin (*M. longissimus lumborum*). The short-loin from 79 red (18 hinds and 20 stags) and wapiti-red crossbred (20 hinds and 21 stags) deer between the ages of 12 and 14 months at slaughter was subject to either 3d or 42d aging treatment at 1 ± 1°C prior to freezing. After thawing, the sarcomere length, ultimate pH (pH_{ult}), water holding capacity, colour and Warner-Bratzler shear force parameters were measured. NIR spectra were collected after meat had been frozen and thawed a second time. Reference meat quality measures and spectra from both aging times were combined and split into calibration (75%) and validation (25%) datasets to evaluate NIR predictive ability using type 1 partial least squares regression. NIR was able to predict pH_{ult} with enough accuracy (R^2_{pred} = 66%, SE_{pred} = 0.10) to identify samples where $pH_{ult} \geq 5.80$, and to identify 5 out of 7 samples where peak shear force was ≥ 8.00 kgF. R^2_{pred} for Warner-Bratzler peak force was low (27%, SE_{pred} = 1.55 kgF). Inconsistent performance between cross-validation and prediction for several traits suggests that cross-validation is not necessarily indicative of future predictive ability. Further work is needed to determine whether NIR spectra from fresh (never frozen, un-aged) venison collected under abattoir conditions can be used to predict venison meat quality parameters.

8.1 Introduction

For meat consumers, a positive meat eating quality experience increases the likelihood of repeat purchases (Grunert 2005). Venison has a low fat content and high iron content (Drew and Seman 1987; Elliot 1993; Purchas *et al.* 2010) which adds to its perception as a high-value meat in many markets (Hoffman and Wiklund 2006). A survey of 276 venison consumers undertaken in the UK found that the 63% of the respondents cited taste and flavour as their main reason for consuming venison, while 35% of respondents stated the main reason they consume venison was its low fat content (Davies 2009). The saleable meat yield and the quality of the meat are important determinants of the deer carcase value. Despite this, meat quality is not currently part of the carcase evaluation system for venison. The ultimate arbiters of meat eating quality are the consumers, but for reasons of cost and practicality, a number of technological methods are used to assess meat quality, such as slice shear force as a proxy for tenderness (Shackelford *et al.* 1999b), and measures of meat colour, ultimate pH (pH_{ult}) and cooking loss. Measurements such as slice shear force, and cooking loss require destruction of the sample. Measurements of pH and colour are time consuming and labour intensive. So like sensory analysis, current instrumental methods are not ideal for measuring meat quality under abattoir conditions and are unlikely to play a direct role in venison carcase evaluation.

Deer are known to be susceptible to pre-slaughter stress (Jago *et al.* 1993; Pollard *et al.* 2002; Pollard *et al.* 2003). Pre-slaughter stress leads to depletion in cellular glycogen reserves, the meat from deer slaughtered in this state will enter rigor before the pH has dropped below 5.8 (Stevenson-Barry *et al.* 1999a). Venison that has a high pH_{ult} is dry, firm and dark (DFD), and has a poorer shelf life (Wiklund *et al.* 2004). Venison with ultimate pH_{ult} between 5.80 and 6.20 is also likely to be tougher than meat with a normal pH_{ult} due to a curvilinear relationship between shear force and pH_{ult} (Stevenson-Barry *et al.* 1999a) (also see Figure 7.1). The incidence of high pH_{ult} in venison can be high, one report on 3856 deer carcases slaughtered at a New Zealand slaughter plant over a 40 day period, found that 18.5% of carcases had shoulder muscles with $pH_{ult} > 5.80$, 10.6% loin muscles with $pH_{ult} > 5.80$ and 5.6% with leg $pH_{ult} > 5.80$ (Pollard *et al.* 1999).

Visible-near infrared (NIR) spectroscopy in combination with multivariate calibration and prediction phases has been identified as a suitable technology to predict meat quality parameters in a fast, non-destructive, safe and cost-effective manner (Osborne *et al.* 1993). These properties make NIR appealing for routine carcase evaluation and determination of meat quality. The mechanics, theory and analysis of NIR spectra are discussed in Section 2.7. Many researchers have applied NIR to predict sensory and technological parameters of meat quality with varying degrees of success (Prevolnik *et al.* 2004; Andrés *et al.* 2007; Prieto *et al.* 2009b; Shackelford *et al.* 2012b). A summary of previous research where NIR has been applied to predict instrumental meat quality in beef and lamb is provided in Table 2.15, Table 2.16 and Table 2.17, but there are no published reports detailing the performance of NIR when applied to predict instrumental meat quality parameters of venison.

The aim of the current experiment was to:

- Determine whether NIR spectra can be used to predict instrumental meat quality parameters of venison *M. longissimus lumborum.*

8.2 Materials and methods

8.2.1 Animals, abattoir and meat quality assessment

Details of the 79 deer used in this experiment are given in Section 7.2.1. The abattoir protocol and the recovery of *M. longissimus lumborum* (LL) short-loin samples are described in Section 7.2.2. The short-loin was halved laterally, vacuum packaged, and each half assigned either to a 3d or 42d aging treatment at 1 ± 1°C. Meat quality data were available on 78 animals at two aging times resulting in a total of 156 records. Purge was calculated by subtracting the weight of the LL sample upon opening from the weight at packing and expressing the difference as a percentage of the packing weight. Meat quality assessment is outlined in Section 7.2.3. For Warner-Bratzler shear force, a 25 mm steak from the middle of the shortloin was cooked for 90 minutes at 70°C within plastic bags that were suspended in a water bath (Purchas and Aungsupakorn 1993). Ten replicate shears were performed per sample, parameters recorded for each shear were the average initial yield force (IYF), the average Warner-Bratzler peak shear force (WBSF), and the average shear force through the duration of the shear as an index of

the work done (WD) (Purchas and Aungsupakorn 1993). WBSF – IYF was also calculated as the difference between the peak shear force and the initial yield has been linked to the connective tissue component of meat toughness (Beilken *et al.* 1986). A ~15 mm steak from the posterior end of the short-loin was retained for NIR spectroscopy and re-frozen in sealed plastic bags at –30°C for at least one week. The remaining anterior section of the short-loin was used for pH_{ult}, sarcomere length (SL) and colour measurements, Lightness (L*) redness (a*) and yellowness (b*) as described in Section 7.2.3.

8.2.2 NIR spectra collection

The posterior part of the short-loin was transported in a frozen state to AgResearch-MIRINZ, Ruakura for the NIR analysis. Two samples were missing, so 154 samples were defrosted for 8 hours at ambient temperature. Samples were "butterflied" to expose one large surface for NIR scanning (Figure 8.1) and allowed to bloom for 2 minutes before scanning as recommended by Shackelford *et al.* (2005). Four replicate spectra (350-1830nm) were collected using a QualitySpec BT (ASD Inc., Boulder Colorado) NIR spectrometer after rotating the sample 90 degrees between scans. The QualitySpec BT is designed for on-line scanning of beef carcases so a polystyrene pedestal was used to mount the venison sample for scanning (Figure 8.1).

Figure 8.1 A sample of venison short-loin being "butterflied" (left) the venison sample on a polystyrene pedestal for scanning (centre and right). The sample was rotated 90 degrees between four replicate scans.

8.2.3 Pre-processing of NIR spectra

Spectra were recorded as absorbance log (1/Reflectance). Some preliminary analysis determined that taking the average or median value at each wavelength resulted in similar predictive ability (results not shown), the median was used here as it is less susceptible to extremes. The median value at each wavelength was calculated from the four replicates, and was used to form the final spectral reading for each meat sample. Plotting median spectra revealed that regions at the extremes of the range (350-1830 nm) contained excessive noise (Figure 8.2). Removing these sections (350 to 559 nm and 1601-1830 nm) resulted in 560-1600 nm as the working spectra (Figure 8.3).

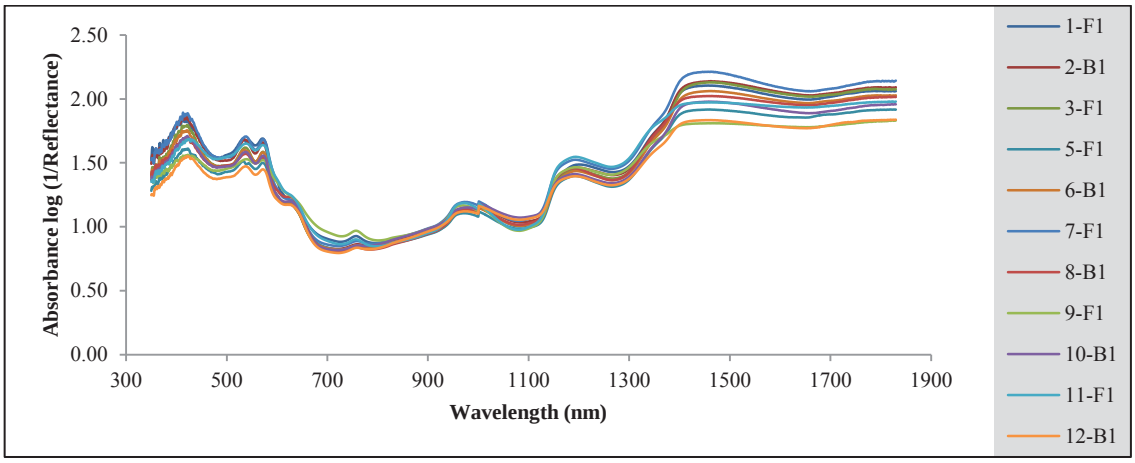

Figure 8.2 Median spectra for 12 samples over the full range (350-1830 nm), excessive noise is visible at the lower end of the spectral range; noise is also present at the upper end of the spectral region but is not visible at this resolution.

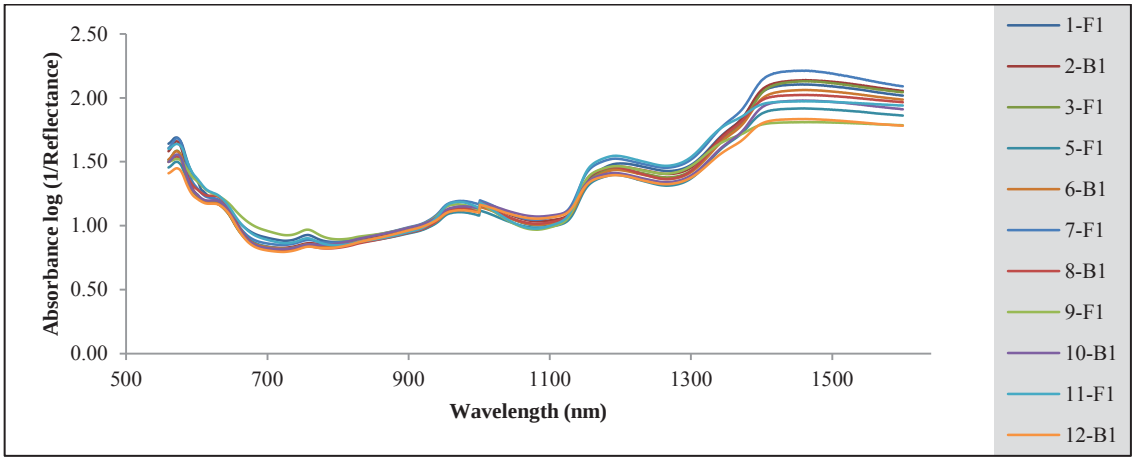

Figure 8.3 The working spectral range (500-1600 nm) for median spectra after removing excessive noise.

Scatter effects resulting from interactions between light and structural properties of the sample (such as particles or droplets) (Osborne *et al.* 1993) were visualized by plotting each individual spectra against the average of all spectra (Geladi *et al.* 1985). Additive effects are seen as different y-axis offsets for different spectra while multiplicative scatter effects are seen as peak intensity dependant spread between different spectra (Esbensen *et al.* 2009). Mostly multiplicative scatter effects were present in the median spectra from venison (Figure 8.4) so multiplicative scatter correction (MSC) was identified as a suitable pre-treatment (Figure 8.5). The details of the MSC transformation procedure are given in Esbensen *et al.* (2009).

8.2.4 Analysis of NIR data

The reference meat quality data and the NIR spectra from both 3d and 42d aged samples were combined in to one dataset to in order to create a large enough dataset to split into calibration and prediction phases. It is important to note that the characteristics responsible for variation in a meat quality parameter may be different at each aging time. For example, the contribution of myofibrils to toughness will be greater at the 3d aging period compared to the 42d aging period (Koohmaraie *et al.* 1991).

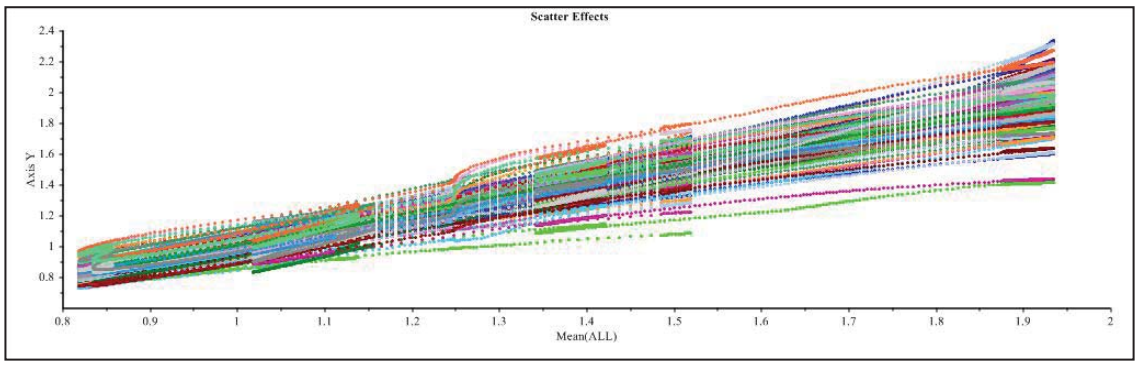

Figure 8.4 A plot of individual median spectra against the average of all spectra, the apparent fanning of the spectra indicates there are multiplicative scatter effects.

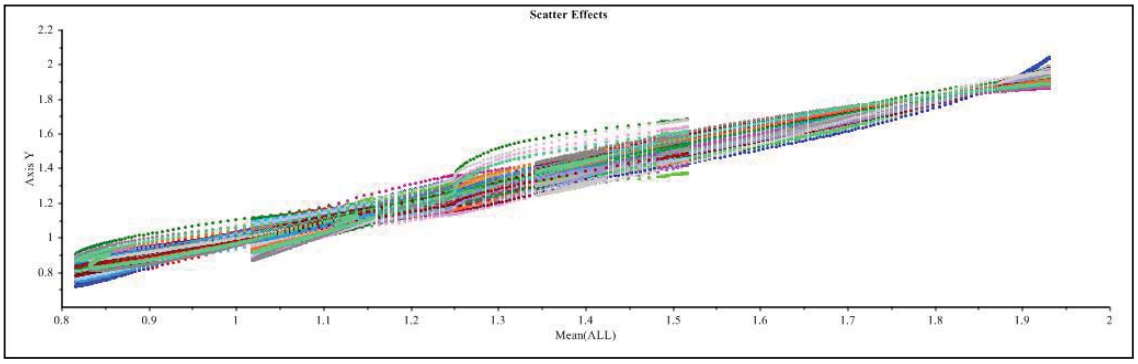

Figure 8.5 MSC-transformed median spectra plotted against the average of all spectra. The plot shows that the MSC pre-treatment has removed the multiplicative scatter effects.

Partial least squares regression type 1 was used for calibrating and predicting instrumental meat quality traits on venison LL using median NIR spectra (560-1600 nm) collected on each sample as explanatory variables. All analysis was performed using Unscrambler (version 10.1) multivariate analysis software (Camo Software AS. Oslo, Norway). Due to the fact that that the variation in a meat quality parameter may be different at each aging time, a calibration and cross-validation analysis was undertaken for each venison quality parameter within each aging time as well as between both aging times. The descriptive statistics (Table 13.1) and performance based on calibration and cross-validation (Table 13.2) within and between each aging time are included in the Appendix (Chapter 13), but will not be considered further in this analysis. It was decided that combining the data from both aging times would facilitate the development of models that are applicable to both aged and un-aged venison samples and would create a dataset large enough to be split into calibration and prediction parts. In order to split samples into calibration and prediction datasets, the samples were sorted in ascending order separately for each parameter and every fourth sample was assigned to the prediction dataset, with the intervening three samples being allocated to the calibration dataset as recommended by Williams (2001). As a result, the samples assigned to each dataset are dependant on the trait of interest, thus the prediction dataset is representative of the calibration dataset with a similar mean, standard deviation and range to the calibration dataset. The prediction dataset is only used for testing the model as recommended by Naes *et al.* (2002). The calibration model was then subject to full leave-one-out cross-validation where each sample is removed, predicted, and replaced in a sequential manner (Naes *et al.* 2002). Performance is gauged with the coefficient of determination and standard error for the calibration (R^2_{cal},

SE_{cal}), cross validation (R^2_{cv}, SE_{cv}) and prediction R^2_{pred}, SE_{pred}).The method for the detection of outliers is described in Section 4.2.7 on page 92.

Because prediction ability is dependant on the variation in the raw data of the trait to be predicted, the ratio of performance deviation (RPD) which is the standard deviation of the Y variable in the calibration dataset divided by SE_{cv} (RPD_{cv}) or, preferably, the standard deviation of the Y variable in the prediction dataset divided by the SE_{pred} (RPD_{pred}) (Cozzolino *et al.* 2000; Williams 2001; Prieto *et al.* 2009a; Agelet and Hurburgh 2010). A higher SD would result in a higher RPD. An RPD above 8 indicates the model is excellent and can be used with confidence whereas RPD values below 2.3 indicate a very poor model and application is not recommended. If the $SE_{pred/cv}$ is similar to the SD of the reference data (RPD ≈ 1.00), the instrument is not predicting the reference data. (Williams 2001).

8.3 Results and discussion

Descriptive statistics for the calibration and prediction dataset are shown in Table 8.1. The means are similar between the datasets although there are some differences in the standard deviations (SD) between the two data sets.

Table 8.1 Descriptive statistics for the calibration and prediction datasets for venison.

	Calibration				Prediction			
Parameter	*n*	Mean	SD	Range	*n*	Mean	SD	Range
Ultimate pH (pH_{ult})	116	5.60	0.17	5.41-6.31	38	5.59	0.16	5.42-6.2
Purge (%)	116	3.92	1.61	0.00-8.85	38	3.87	1.52	0.76-7.32
Expressed juice (EJ) (cm^2g^{-1})	114	29.38	3.71	21.51-39.10	37	29.33	3.45	22.48-36.46
Cooking loss (CL) (%)	116	28.09	2.03	21.76-32.19	38	28.06	1.95	22.34-31.12
Sarcomere length (SL) (µm)	116	1.57	0.10	1.23-2.00	38	1.57	0.08	1.30-1.71
Lightness (L*)	116	36.34	2.37	29.66-41.12	38	36.30	2.29	30.25-39.87
Redness (a*)	116	12.01	1.55	6.87-14.86	38	12.00	1.47	8.59-14.60
Yellowness (b*)	116	3.11	0.80	0.92-4.75	38	3.10	0.78	1.32-4.58
WBSF[a] (kgF)	116	6.43	2.04	3.13-13.07	38	6.35	1.90	3.31-10.61
Initial yield force (IYF) (kgF)	116	5.54	1.84	2.64-11.57	38	5.48	1.74	2.77-9.63
WBSF – IYF (kg)	116	0.88	0.50	0.15-2.58	38	0.87	0.47	0.19-2.10
Work done (WD)	116	1.99	0.56	1.00-3.48	38	1.98	0.54	1.02-3.27

[a] WBSF = Warner-Bratzler peak shear force.

8.3.1 Prediction of ultimate pH

The R^2_{cal} = 79% (SE_{cv} = 0.10) (Table 8.2) is lower than the R^2_{cal} of 97% (SE_{cv} = 0.10) reported by Andrés *et al.* (2008) on *M. longissimus thoracis* (LT) from 30 bulls, and similar to R^2_{cal} = 88%, SE_{cv} = 0.14) obtained in Section 4.3.5 on beef, where the high correlation was largely due to bulls having a higher pH_{ult} than steers and heifers. There is further similarity between the current results and the R^2_{cal} values of 81% (SE_{cv} = 0.18) obtained on 100 beef LT samples reported by Cozzolino and Murray (2002) and 85% (SE_{cv} = 0.20) obtained on LT from 26 Hereford steers (Rosenvold *et al.* 2009). Prieto *et al.* (2008) reported an R^2_{cal} of 41%, SE_{cv} = 0.06, RPD_{cv} = 1.12 for LT pH_{ult} on 53 steers and Lomiwes (2008) reported an R^2_{cv} of 20%, (SE_{cv} = 0.13) for pH_{ult} on 85 beef carcases, but NIR spectra were collected on pre-rigor beef in the latter study. In the current study, NIR spectra were able to predict pH_{ult} with an R^2_{pred} = 66% (SE_{pred} = 0.10, RPD = 1.63). The model was able to correctly identify the three samples in the calibration dataset with pH_{ult} values > 5.80, but misclassified one sample with a normal pH_{ult} as having a pH_{ult} > 5.80 (Figure 8.6).

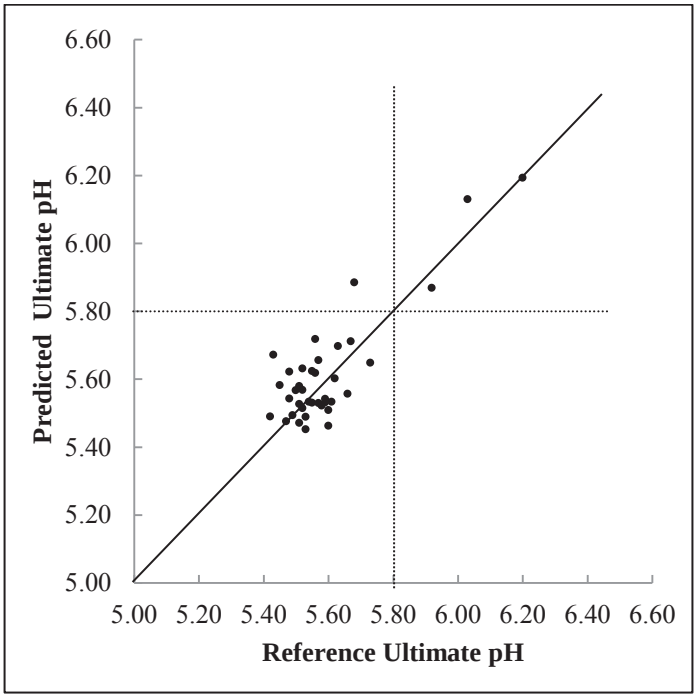

Figure 8.6 Prediction of pH_{ult} from NIR spectra on 38 *M. longissimus lumborum* samples showing that the model could correctly identify the three samples with $pH_{ult} \geq 5.80$.

Table 8.2 Performance of NIR calibration equations showing the coefficient of determination (R^2) and standard error (SE) for calibration, cross-validation and prediction phases for predicting instrumental meat quality parameters on venison short-loin.

Parameter	Pre-Treatment[a]	PC[b]	Calibration			Cross-validation			Prediction			
			n[c]	R^2	RMSE[d]	R^2	SE	RPD[e]	*n*[f]	R^2	SE	RPD[g]
Ultimate pH (pH_{ult})	MSC	10	116	78.8	0.08	68.0	0.10	1.75	38	66.4	0.10	1.63
Purge (%)	none	2	112	19.4	1.35	15.0	1.41	1.15	37	29.6	1.30	1.18
Expressed juice (EJ) (cm^2g^{-1})	MSC	0	116	35.0	2.98	13.5	3.48	1.07	36	34.5	2.67	1.23
Cooking loss (CL) (%)	MSC	3	116	21.5	1.79	16.2	1.88	1.08	38	na[h]	na	na
Sarcomere Length (SL) (µm)	none	2	115	17.4	0.08	11.4	0.08	1.15	38	36.7	0.06	1.28
Lightness (L*)	none	4	116	40.4	1.82	33.2	1.95	1.21	38	8.3	2.42	0.94
Redness (a*)	none	4	115	42.8	1.15	35.1	1.24	1.25	38	62.3	0.92	1.60
Yellowness (b*)	none	3	115	53.4	0.54	50.0	0.57	1.40	38	40.4	0.59	1.31
Warner-Bratzler peak shear force (WBSF) (kgF)	none	3	114	54.5	1.31	51.6	1.37	1.49	34	27.0	1.55	1.20
Initial yield force (IYF) (kgF)	none	3	110	39.0	1.6	35.0	1.42	1.29	37	54.4	1.16	1.50
WBSF – IYF (kg)	none	3	116	45.4	0.37	40.6	0.39	1.29	38	50.8	0.32	1.47
Work done (WD)	none	4	112	48.7	0.39	44.4	0.48	1.15	38	50.0	0.40	1.35

[a] Pre-treatment indicates whether multiplicative scatter correction (MSC) has been applied to the spectra prior to analysis.
[b] PC = number or principal components used in the regression.
[c] *n* = number of samples included in calibration and cross-validation phases.
[d] RMSE = root mean square error.
[e] RPD = ratio performance deviation is the SD of the Y variable in the calibration dataset (after removal of outliers) divided by the SE_{cv}.
[f] *n* = number of samples used for the prediction phase.
[g] RPD = ratio of performance deviation (SD of the prediction dataset divided by the SE of prediction).
[h] na = not available, the prediction phase failed for cooking loss so no results were obtained.

8.3.2 Purge, expressed juice and cooking loss

In order to develop a calibration equation for purge using NIR spectral data, the minimum and maximum values were removed, the minimum value was 0% purge and was likely to be an error, the maximum value (8.86%) was > 3 SD from the mean and could be justified as an outlier. Two further samples with spectra that were significantly different from the mean spectra of the population ($P < 0.01$) were also removed from the analysis due to poor performance in the calibration phase. One sample was also removed from the prediction dataset based on poor spectra ($P < 0.01$). Retaining this sample in the prediction reduced the R^2_{pred} to 24%, SE_{pred} = 1.31). There are no reports where NIR has been applied to predict vacuum package purge as reported here where R^2_{pred} = 30%, SE_{pred} = 1.30, RPD_{pred} = 1.18), (Table 8.2. Leroy *et al.* (2004) analysed purge in a plastic bag on LT samples from 88 bulls and reported R^2_{cv} = 51% (SE_{cv} = 0.51%, RPD_{cv} = 1.40) for 2d-aged samples and R^2_{cv} = 54% (SE_{cv} = 0.82%, RPD_{cv} = 1.46) for 8d-aged samples. Prieto *et al.* (2008) reported R^2_{cal} = 26% (SE_{cv} = 0.36, RPD = 1.04) for drip loss using the Honikel bag method (Honikel 1998) on LT samples from 53 steers and R^2_{cal} = 20% (SE_{cv} = 0.55, RPD_{cv} = 1.02) on 67 young cattle.

Expressed Juice is a measure of water holding capacity using the filter paper press method based on that of Hamm (1986). EJ could be predicted with an R^2_{pred} = 34.5%, SE_{pred} = 2.67 cm^2g^{-1}, RPD_{pred} = 1.23 (Table 8.2). One sample was removed from the prediction dataset due to having anomalous spectra ($P < 0.001$). Retaining this sample reduced the R^2_{pred} to 23% (SE_{pred} = 3.07). Prieto *et al.* (2008) reported an R^2_{cal} of 48% (SE_{cv} = 2.08, RPD_{cv} = 1.11) for prediction of EJ on 53 steers and R^2_{cal} = 58% (SE_{cv} = 2.51, RPD_{cv} = 1.30) on the LT of 67 young cattle. Ripoll *et al.* (2008) obtained a R^2_{pred} of 89.2 (SE_{pred} = 1.34%, RPD = 1.76) for EJ on LT from 190 bulls using calibrations equations developed on 75% of the samples and applied to the remaining 25% of the samples. In a different study involving LT from 40 Hereford steers, Rosenvold *et al.* (2009) reported R^2_{pred} = 67% (SE_{pred} = 2.8 cm^2g^{-1}) for EJ. The NIR spectra in the studies of Prieto *et al.* (2008) and Ripoll *et al.* (2008) were collected on homogenised samples, but Rosenvold *et al.* (2009) collected spectra and EJ reference measures on intact meat over a range of aging times so is the most similar to the current analysis. Despite being able to develop and cross-validate a prediction equation to predict

cooking loss, the model completely failed to predict cooking loss in the prediction dataset. It can be seen that cross-validation is not indicative of prediction on new samples in this case. Leroy *et al.* (2004) reported an R^2_{cal} of 25% (SE_{cv} = 2.31%, RPD_{cv} = 1.13) on 101 cow and 88 bull LT samples aged for two days prior to cooking. Andrés *et al.* (2008) reported an R^2_{cal} of 20% (SE_{cv} = 0.08%, RPD_{cv} = 1.01) on LT samples from 30 bulls. Prieto *et al.* (2008) reported an R^2_{cal} of 14% (SE_{cv} = 1.61%, RPD_{cv} = 1.03) on LT samples aged seven days from 53 oxen and R^2_{cal} of 0.001% (SE_{cv} = 2.45%, RPD_{cv} = 0.97) on LT aged for three days from young cattle, although the NIR spectra were collected on homogenised meat samples as opposed to the intact LT muscle, Prieto *et al.* (2009b) reported an R^2_{cal} of 35% (SE_{cv} = 2.35%, RPD_{cv} = 1.14) on 130 LT samples aged 14d, but 64 samples were excluded from the analysis so this value may be optimistic if the whole dataset were used. It is possible that the poor prediction ability of NIR for cooking loss is a result of heterogeneity in the samples, possibly due to fat forming a barrier to cooking loss (Hornstein *et al.* 1960), or the fact that smaller carcases yield smaller muscle samples at a constant thickness which have a higher surface area to volume ratio resulting in greater cooking loss. This is likely to be of importance when the entire slice of LL is cooked without any standardization of dimensions which was the case in the current analysis.

8.3.3 Prediction of sarcomere length (SL)

After removal of one sample (where the reference measure of SL was > 4 SD from the mean) from the calibration dataset, a prediction equation was developed that performed better in the prediction phase (R^2_{pred} = 36.7%, SE_{pred} = 0.06 µm, RPD_{pred} = 1.28) than the cross-validation (R^2_{cv} = 11.4%, SE_{cv} = 0.08 µm, RPD_{cv} = 1.15) (Table 8.2). Removing scatter effects reduced performance (data not shown) which suggests that variation in sarcomere length may be responsible for some of the scatter effects in the sample (Cozzolino and Murray 2002). Rødbotten *et al.* (2001) found that the absorbance spectra differed between samples with long (> 2.0 µm) and short (< 1.6 µm) sarcomeres in the spectral region below about 1150 nm, shorter sarcomeres had a higher absorption than long sarcomeres in LT samples from 12 young bull carcases. Rødbotten *et al.* (2001) reported a highly a significant correlation between Warner-Bratzler shear force and sarcomere length (r = –0.67, P < 0.001) and postulated that the differing absorption patterns associated with shortened sarcomeres may underpin the prediction

of Warner-Bratzler shear force, but they did not attempt to predict sarcomere length directly. In the current analysis, the correlation between Warner-Bratzler peak shear force and sarcomere length was –0.35, (P = 0.002) for 3d aged samples and –0.48 (P < 0.001) for 42d aged samples (data not shown). There are few reports where NIR spectra have been applied to predict sarcomere length, Andrés *et al.* (2008) reported a R^2_{cal} = 16% (SE_{cal} = 0.08 µm) and R^2_{cv} = 2% (SE_{cv} = 0.10 µm, RPD_{cv} = 0.84) for LT SL on a sample of 30 young Maronesa bulls. Shackelford *et al.* (2012a) reported that the mean sarcomere length was significantly shorter in LT classified "not tender" compared to LT classified tender, although they did not predict or classify samples based on sarcomere length *per se*. Shackelford *et al.* (2012a) postulated that the biochemical basis for classifying LT into tenderness classes is sarcomere length and post-mortem proteolysis based on the percentage of desmin degradation, If this is the case, prediction of sarcomere length and the levels of desmin directly using NIR spectral data would help to verify this.

8.3.4 Prediction of venison colour

No spectral pre-treatments were deemed necessary for prediction of colour traits which is in agreement with the analysis on beef (Section 4.3.4) and with the prediction of lightness (L*) and redness (a*) of lamb (Section 6.3.2). Prieto *et al.* (2009b) also found that no pre-treatments were necessary for prediction of beef colour. This consistency suggests that the scatter effects are informative for the prediction of meat colour, removing scatter effects reduced model performance (results not shown). Prediction of redness (R^2_{pred} = 62.3, SE_{pred} = 0.92, RPD_{pred} = 1.60) and yellowness R^2_{pred} = 40.4, SE_{pred} = 0.598, RPD_{pred} = 1.31) (Table 8.2) was much better than lightness R^2_{pred} = 8.3, SE_{pred} = 2.42, RPD_{pred} = 0.94). The poor performance for prediction of lightness was surprising given that calibration and cross-validation phases were much stronger (RPD_{cv} = 1.21). The descriptive statistics for lightness were similar for both the calibration and prediction datasets, so prediction performance should have been similar to the cross validation performance. The reduction in performance was not due to spectral or reference outliers in the prediction dataset, removing suspect samples did not offer any large improvements in accuracy (results not shown). One possible explanation is that the cut-off of 559 nm used to eliminate excessive noise from the lower end of the spectra removed informative wavelengths for predicting lightness, but reducing the

threshold to 399 nm did not improve performance (results not shown). Andrés *et al.* (2008) reported an R^2_{cv} = 75% (SE_{cv} = 1.36, RPD_{cv} = 2.07) for L*, R^2_{cv} = 29% (SE_{cv} = 1.28, RPD_{cv} = 0.90) for redness and R^2_{cv} = 46% (SE_{cv} = 0.99, RPD_{cv} = 1.37) for yellowness for 30 bull LT samples after allowing the meat to bloom for 60 minutes. Prieto *et al.* (2009b) obtained R^2_{cv} = 83% (SE_{cv} = 0.96, RPD_{cv} = 2.47) for L*, R^2_{cv} = 76% (SE_{cv} = 0.95, RPD_{cv} = 2.02) for a* and R^2_{cv} = 69% (SE_{cv} = 0.84, RPD_{cv} = 2.48) for b* for beef LT samples after allowing the meat to bloom 45 minutes. An R^2_{pred} = 82%, SE_{pred} = 1.18, RPD_{pred} = 2.35 was obtained for lightness on 59 beef LT samples in Section 4.3.4.

8.3.5 Prediction of venison shear force parameters

After removal of two samples, one due to anomalous spectra ($P < 0.005$) and the maximum record (13.07 kgF) which was > 3 SD from the mean peak shear force value. Strong calibration (R^2_{cal} = 54.5), and cross-validation (R^2_{cv} = 51.6, SE_{cv} = 1.37) performance for WBSF did not translate into such strong prediction performance (R^2_{pred} = 27%, SE_{pred} = 1.55 kgF, RPD_{pred} = 1.20) (Table 8.2). The reason for the reduction in performance could not be determined, as removal of outliers failed to improve predictive ability, yet this result shows that cross-validation is not always a good indicator of future prediction performance. Twelve studies on beef have used NIR spectra to predict Warner-Bratzler peak shear force (for details see Table 2.15, Table 2.16 and Table 2.17). The average RPD_{cv} values reported for these 12 studies for LL WBSF is 1.20 (RPD_{cv} ranging from 1.05 to 1.46), the results of Park *et al.* (1998) were excluded because carcases were selected based on WBSF values to maximize variation. This average RPD_{cv} value is lower than the RPD_{cv} = 1.49 but the same as the RPD_{pred} = 1.20 obtained in the current analysis for WBSF in venison. In contrast, the prediction for initial yield force was much stronger than the cross validation performance (Table 8.2). Six outliers from the calibration dataset and two samples from the prediction dataset were removed, retaining outliers reduced the R^2_{cv} to 25% (SE_{cv} = 1.60) and R^2_{pred} to 26.0% (SE_{pred} = 1.52) (data not shown). For the both the calibration and the full datasets, the correlation between WBSF and IYF is very high (r = 0.97, $P < 0.001$), which is in agreement with the r = 0.98, $P < 0.001$) reported by Peachey *et al.* (2002) in beef. It is not clear why the prediction performance was stronger for IYF than for WBSF. Performance was also stronger in the prediction phase for WBSF – IYF and

WD. WBSF – IYF has previously been associated with the collagen component of meat (Beilken *et al.* 1986). In some early work on NIR applied to lamb meat, Young *et al.* (1996) reported that NIR spectra from intact samples of lamb *M. semimembranosus* were correlated to the percentage of soluble collagen with a correlation (r) of 0.88, SE_{cv} = 1.19%, but Downey and Hildrum (2004) state that NIR spectrum of collagen is very similar to that of myofibrillar proteins, which are present in 10 times higher concentrations than collagen in meat. Collagen cross-linking increases with animal age and results in reduced collagen solubility causing increasing toughness (Young and Braggins 1993). The percentage of soluble collagen may be the biological mechanism underpinning this relationship, but the age range of the deer used in this experiment was very low (12-14 months), so it is unlikely that there would be much variation in collagen solubility.

Four outliers were removed from the calibration phase for WD, but no samples were removed from the prediction phase. Because WD was highly correlated (r = 0.95, P < 0.001) to WBSF, the samples that have a high WBSF also have a high WD (Figure 8.7). Bickerstaffe *et al.* (2001) used ≥ 8.00 kgF as a threshold for classifying lamb samples as tough based on the MIRINZ shear force test. Davies *et al.* (2009) also used ≥ 8.00 kgF as a threshold for classifying venison samples as "very tough" using the Volodkevitch shear force test. It should be noted that the relationship between different shear force instruments may not be linear across the entire range of toughness encountered in lamb meat (Hopkins *et al.* 2011b). Therefore inferring consumer tenderness thresholds based on one shear force instrument may not be fully applicable to other shear force tests, but Peachey *et al.* (2002) reported a correlation of 0.93 (P < 0.001) between WBSF and MIRINZ peak shear force so the high correlation suggests that this threshold could reasonably be applied to WBSF as used in the current analysis. In that experiment, a MIRINZ peak shear force of 8.00 kgF was equivalent to a WBSF ≈ 12.00 kgF, so the threshold of 8.00 kgF is conservative. For both WBSF – IYF and WD, prediction performance was better than calibration / cross-validation performance (Table 8.2). The WD of a sample is easier to determine than IYF from a Warner-Bratzler force deformation curve, so using the model for WD, it was possible to identify 5 out of 7 samples where WD exceeded 2.50 (Figure 8.8) which also correctly identified 5 out of 7 samples where WBSF was ≥ 8.00 kgF (Figure 8.7 and Figure 8.8). Interestingly, all 7 samples where WD ≥ 2.50 came from stags, and in the current analysis, LL from stags

had a higher WD than hinds (Table 7.5), which is consistent with the results reported by Purchas *et al.* (2010) who reported that LL from stags had a higher WD than that from hinds.

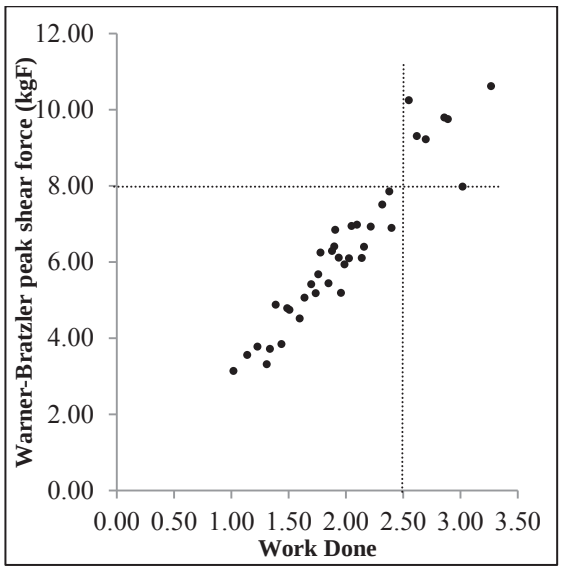

Figure 8.7 A plot showing the correlation (r = 0.95, P < 0.001) between Warner-Bratzler peak shear force and work done (the average force throughout the shear) for the prediction dataset. Samples where WD is ≥ 2.50 also have a WBSF ≥ 8.00 kgF.

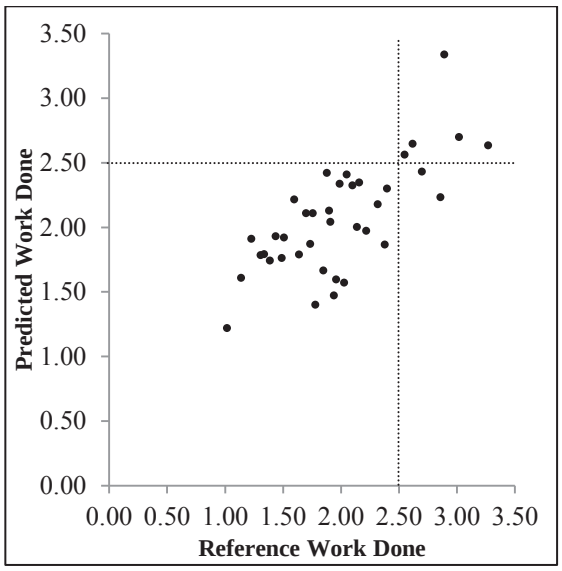

Figure 8.8 A plot showing WD predicted from NIR spectra against the reference WD for the prediction dataset. A threshold of 2.50 can be used to identify samples where WBSF is ≥ 8.00 kgF.

8.3.6 Future directions for using NIR to predict venison meat quality

NIR spectra were collected on venison samples that had been aged for either 3 or 42d and that had been frozen prior to scanning. If NIR spectra were to be used to predict meat quality in the processing plant, scanning would need to be performed on fresh (un-aged and never frozen) meat. Freezing and thawing is known to alter the NIR spectra of beef (Downey and Beauchêne 1997; Thyholt and Isaksson 1997). It is possible that spectra from frozen then thawed venison would also differ from spectra of fresh venison; so further research is needed to establish the relationship between NIR spectra recorded on fresh venison and venison meat quality.

8.4 Summary and conclusions

1. Although the characteristics responsible for variation in a meat quality parameter may be different at each aging time, models were developed to predict venison quality parameters that were applicable to both aged and un-aged venison.

2. There were no previous reports detailing the relationship between NIR spectra and venison meat quality so comparisons have been made with reports on beef and lamb.

3. NIR was particularly strong for predicting pH_{ult} of venison, samples with a high pH_{ult} can be identified using NIR.

4. Prediction of purge (RPD_{pred} = 1.18) and expressed juice using NIR spectra resulted in RPD_{pred} values of a similar magnitude to those reported in beef (Mean RPD_{cv} = 1.23 (range 1.02-1.46).

5. Cooking loss could not be predicted despite showing some predictive ability in the cross-validation phase. This may be because reference measures of cooking loss are likely to be affected by higher surface area to volume ratios in samples from smaller carcases.

6. Prediction of sarcomere length (RPD_{pred} = 1.28) was better than expected given the poor performance obtained in the cross-validation phase.

7. Prediction of lightness was poor (RPD_{pred} = 0.94) considering that performance was much better in the calibration and cross-validation phases.

8. Prediction of peak shear force (RPD_{pred} = 1.20) was poorer than expected from cross-validation performance, but prediction of IYF (RPD_{pred} = 1.50), WBSF–IYF (RPD_{pred} = 1.47) and WD (RPD_{pred} = 1.35) was stronger than expected.

9. The inconsistent performance between cross-validation and prediction for several parameters indicates that cross-validation is not necessarily indicative of future predictive performance and that a separate prediction group should be included in any NIR evaluation.

10. Due to a high correlation between WBSF and WD, the WD model could be used to identify venison samples where the WBSF was ≥ 8.00 kgF, a threshold that has been used previously to identify tough samples.

11. Future research is needed to determine whether these models would be applicable to spectra obtained under abattoir conditions from fresh (never frozen) venison.

9 General discussion

9.1 Introduction

Overall, the value-chains of beef, lamb and venison from farm to fork are broadly similar. Farmers produce animals to sell to meat processors who add value to the carcase by de-constructing the carcase, conditioning parts of it, de-boning and preparing the meat into consumer-ready packs. The processors then sell meat to retailers where consumers purchase it for consumption.

There are a series of transactions that take place between members of the value chain. The first is the sale of finished animals to the meat processor and is based around an accepted mode of carcase evaluation. Depending on the country and species, carcase evaluation varies but it is supposed to be consistant across the EU. Evaluation systems generally endeavour to determine the value per unit weight of the carcase, but usually do not directly incorporate meat quality parameters so there is no direct flow of information to the producer relating to meat quality. The second transaction occurs between the processor and the retailer where the value per unit weight, ceteris paribus, is determined by the specification of the product (mainly the type of cut, but branded products and aging times may also play a role). There is more likely to be a flow of consumer information here because customer complaints can result in feedback to processors, but because the traceability of meat to a carcase is generally not possible when carcases are broken down into cuts, it becomes difficult to identify what processes in the value chain, or what aspects of the raw material are resulting in poor product consistency and a loss in repeat purchases.

Given that there are at least two transactions taking place before the consumer purchases a meat product, there are at least two opportunities in the value chain to identify and remove carcases/meat of inferior quality in order to prevent inferior product reaching the end user. The relationship between carcase quality and meat quality is not simple; a higher yield of saleable meat does not necessarily result in a higher quality meat product. Through a series of experiments, the aim of this thesis has been to investigate the relationships between video image analysis (VIA) variables and saleable meat yield (SMY%) of high-value cuts in beef carcases and to investigate the

relationships between visible-near infrared (NIR) spectra and instrumental meat quality parameters in beef, lamb and venison of various genders and genotypes. It should be noted that the emphasis on NIR was driven by its potential as a means of identifying carcases with inferior meat quality so as to improve meat product consistency from a consumer perspective.

Considering eating quality and its consistency from the producer end of the value chain is more difficult and relies on the notion that meat quality can be directly incorporated into carcase evaluation systems and that a member of the value chain is prepared to take responsibility for inferior meat quality. Unless the value chain is fully integrated, it is more likely that measurement of meat quality, with the explicit purpose of removing outliers and improving consistency of table cuts, will be of more value to the meat industry than will any sort of quality-based payment to producers on a carcase basis. Having said that, if eating quality information was routinely recorded and fed back to producers, over time it would be possible for processors to identify which farmers are producing superior quality meat and reward them accordingly.

NIR spectra with multivariate calibration equations can be used for the quantitative prediction of chemical constituents in meat (Weeranantanaphan *et al.* 2011). The predictions are based on the absorbance of electromagnetic radiation at certain wavelengths by CH, NH and OH chemical bonds in the fats and proteins that constitute meat (Osborne *et al.* 1993). The research undertaken in this thesis did not strive to elucidate the biological mechanisms underpinning the relationship between NIR spectra and meat quality. It is expected that biological variation will always be present in the populations and VIA and NIR needs to take account of this. The emphasis here was to evaluate the ability of NIR and VIA technologies to quantify quality differences, rather than to characterize the biochemical basis of variation in carcase and meat quality.

A range of genders, genotypes, aging times and muscles of the three species were investigated primarily to represent the conditions typically encountered in a commercial setting where VIA and NIR technologies may contribute to the carcase/meat evaluation process, but the datasets also enabled the magnitude of these effects to be investigated.
Given the fact that three different species were investigated in the experimental chapters, it is tempting make comparisons between them. Such comparisons are difficult

in the context of this thesis because the experiments were very different in nature, thus it is really only possible to make a few general observations linking the three species. In terms of gender and genotype effects on beef (Chapter 4), lamb (Chapter 5) and venison (Chapter 7) results showed that in general, gender had larger effects on meat quality than genotype, which may be because extreme breed-types were not compared in these experiments. VIA was only investigated in beef (Chapter 3), but the performance of NIR for measuring meat quality was investigated in beef (Chapter 4), lamb (Chapter 6) and venison (Chapter 8) and merits some comparison. Because meat toughness is likely to result in customer complaints, the ability of NIR to identify tough meat is of great interest to industry. NIR showed some promise for predicting shear force in venison, but little promise in beef or lamb. The NIR absorbance profiles looked similar across the three species; but the correlation between shear force and absorbance values at each wavelength in the NIR spectrum was inconsistent between species. The true reason for this cannot be determined from these experiments because different methods were used to measure shear force across the species.

Although the shear force predictions were a little disappointing, the utility of the shear force tests also needs consideration, firstly because the correlation between the various shear force tests and tenderness as assessed by un-trained consumers is not particularly strong, and secondly because toughness is perhaps less of a problem in venison and lamb than in beef, due to the fact that lambs are slaughtered at a lower maturity, and potentially because post mortem proteolysis is known to be more rapid in venison than in beef (Barnier *et al.* 1999; Farouk *et al.* 2007). Furthermore, venison exporters are primarily concerned with water holding capacity and shelf-life attributes of venison as opposed to toughness, which tends not to be a major problem.

9.2 What can VIA and/or NIR currently offer?

Based on the experiment where the ability to predict the SMY% of the sirloin region was assessed (Chapter 3), VIA appears to have lower accuracy for the prediction of SMY% at the cut level (Table 3.6) than at the total carcase level as assessed from the published literature (Table 2.8). VIA could be used to evaluate carcases on the basis of SMY%, but the accuracy would be very low as the distribution of SMY% throughout the carcase is not currently predicted (except indirectly via the EUROP grid). It is worth

noting that VIA has been operating for beef on an industrial scale for a number of years and is at a more advanced state of development in comparison to the use of NIR spectra for predicting meat quality. In terms of predicting meat quality, NIR can identify LL samples with high pH_{ult}, and IMF% (Prieto *et al.* 2011) plus some colour parameters. Models developed in Chapter 4 were not accurate enough to be used for identifying beef LT with high shear force values, but previous research showed that extremely tough samples could be identified in beef (Prieto *et al.* 2009b). Models developed in Chapter 8 showed that it is possible to identify venison samples with high shear force values, but performance was poorer in lamb where models were ineffective in identifying high shear force samples.

The ability to identify extremes (and particularly extremes at the undesirable end of the scale) is a first step to value-based marketing. Cross and Whittaker (1992) defined value-based marketing as a system that sends clear and accurate economic signals from the consumer backward through the value chain. In order for a value-based marketing system to function, a means of identifying the value of individual carcases is essential (Cross and Whittaker 1992). In their present forms, VIA and NIR are not sufficiently well developed to provide the full solution for a value-based marketing system. But, VIA offers greater consistency of classification within and between meat processors and is a useful source of information for livestock breeders (Pabiou *et al.* 2012). The ability to identify extremes in some characteristics (Chapters 4, 6 and 8) shows that already, NIR spectroscopy has the potential to improve product consistency. Over time, identification and removal of extremes at the poor end of the scale should result in a greater number of repeat purchases (Grunert *et al.* 2004).

Measures of the success of programs involving the integration of VIA and NIR to identify differences in overall *value* between individual carcases based on both SMY% and meat quality are awaited with interest, as are the results of the integrated meat eating quality (IMEQ) project currently being undertaken by Quality Meat Scotland and the Scottish Government. The aim of the IMEQ project is to develop an automatic or semi automatic system for estimating pH /temperature, carcase conformation, fat class and subcutaneous fat at commercial processing plant line speed, and to integrate it with the output of imaging technologies (such as NIR) used to estimate meat colour and eating quality (Maltin *et al.* 2012).

9.3 Industry challenges

The research reported in this thesis has assessed the accuracy with which VIA and NIR spectroscopy can be used to predict SMY% and meat quality characteristics, respectively, rather than being concerned with the application of SMY% and meat quality information to a carcase evaluation system *per se*. Some important questions arise when considering the paradigm of value-based carcase evaluation at the processing level and at an industry-wide level. These questions include:

1. How is quality defined? Is the definition consumer focused, retailer focused, or processor focussed? Does quality consider both carcase and eating quality?
2. What constitutes an "inferior" carcase in terms of specific characteristics, and what/who should be considered responsible for its low value, (e.g. production effects, pre-slaughter handling effects or processing effects)?
3. Who takes responsibility for the low value and the associated increased costs or reduced returns?
4. What should meat processors do with "inferior" carcases?
5. Assuming value-based marketing is transparent, how should value be divided throughout the value chain (producer, processor, and retailer)?
6. How should the cost of any new technology be borne?
7. Is there a need for value-based marketing in times of short product supply?
8. Would producers risk being penalized for "inferior" meat quality when they have a low-risk alternative at another processing plant?
9. What is an acceptable level of robustness for the prediction of meat quality?
10. Who owns the information on each carcase and how would the information be disseminated up and down the value chain?

A discussion surrounding the possible answers to these questions is beyond the scope of this thesis, but they are questions that the industry should be considering and discussing ahead of future developments in quality-based carcase evaluation.

10 Summary and conclusions

After reviewing the literature on VIA and NIR (Chapter 2) it was concluded that the ability of VIA to predict the saleable meat yield of the high-value loin region containing the sirloin and fillet cuts required further investigation. It was also concluded that the relationship between NIR spectra and beef meat quality required further investigation on a larger, more heterogeneous range of genders and genotypes. Furthermore, a lack of information in the published literature pertaining to the relationship between NIR spectra and meat quality in lamb and venison was identified.

Results obtained in Chapter 3 on 141 carcases from 6 breed-gender groups (steers, heifers and young bull crossbreds of the Charolais, Limousin and predominantly crossbred dairy breeds) showed that VIA and visual carcase classification systems operating on the EUROP grid had a similar ability to predict the yield of saleable sirloin as a percentage of hot carcase weight. Both classification systems were poor at predicting the yield of fillet. Results also showed that the weight of excess fat trimmed during preparation of the sirloin did not account for any further variation in saleable meat yield of either the sirloin or fillet yield, but the weight of bone removed did account for some additional variation in fillet yield. It was concluded that the relationship between VIA variables and the carcase composition require further investigation.

Results obtained in Chapter 4 on 234 samples of *M. longissimus thoracis* from 6 genotype-gender groups (steers, heifers and young bull crossbreds of the Charolais, Limousin and dairy genotypes) showed that the genotype had minimal effects on meat quality but *M. longissimus thoracis* from young bulls was of poorer quality than that of steers. In addition, the relationship between NIR spectra and beef quality showed that ultimate pH and meat colour could be predicted with the highest levels of accuracy, but shear force was poorly predicted. It was concluded that further analysis is required on shear force using a novel support vector machine regression approach.

In an experiment involving 208 Texel lambs (Chapter 5), the effects of sex on the meat quality of lamb *M. longissimus lumborum* and *M. semimembranosus* meat quality was

investigated. Results showed that sex had some small but significant effects on lamb meat quality after aging meat for between 7 and 9 days. In addition, the Texel muscling quantitative trait locus (TM-QTL) was found to have no significant effects on the meat quality of *M. semimembranosus*. It was concluded that finishing ram lambs to the specifications used in the experiment would result in minor differences in meat quality between ewes and rams. Further investigation is needed to determine whether the effects of TM-QTL and sex would alter if lambs were finished to higher weights or if carcases were aged for a shorter time.

In an experiment involving the 208 lambs from Chapter 5, results (Chapter 6) showed that NIR spectra collected on the lamb from *M. longissimus lumborum* aged for between 7 and 9 days could be used to predict the percentage of intramuscular fat in that muscle and showed some promise for predicting the colour traits in *M. semimembranosus*. Results showed that ultimate pH and shear force of *M. longissimus lumborum* and *M. semimembranosus* was poorly characterized by NIR spectra collected on the *M. longissimus lumborum*. It was concluded that further investigation is needed to determine the relationship between NIR spectra and the quality of meat aged for a shorter time from lambs more typical of the UK slaughter population.

Results obtained from an experiment involving the *M. longissimus lumborum* from 79 deer (Chapter 7) showed that sex, genotype (red or wapiti-red crossbreds), sampling location within the *M. longissimus lumborum* and chilled aging time affected venison meat quality. Meat from stags was tougher (higher shear force values) than that of hinds and meat from wapiti-red crossbreds was tougher than that of red deer. Results also showed that the anterior part of the muscle was tougher than the posterior after 3d aging but such effects were present after an additional 39d chilled aging. Genotype was confounded with farm and slaughter day effects so it was concluded that further research is needed to verify the apparent genotype effects on venison *M. longissimus lumborum* meat quality.

The relationship between NIR spectra and meat quality of frozen then thawed aged and un-aged and venison from the 79 deer in Chapter 7 was characterized in Chapter 8. Results showed that NIR spectra could be used to identify samples with high ultimate

pH and shear force values. NIR spectra were collected on meat samples that had been frozen and thawed twice, so it was concluded that further research would be needed to establish whether the models could be applied to predict meat quality of fresh (un-aged and never frozen) venison under abattoir conditions.

The general discussion (Chapter 9) considered the current utility of VIA and NIR for carcase evaluation based on the findings of the experimental chapters (Chapters 3-8) and the published literature reviewed in Chapter 2. It was proposed that VIA could be used to determine conformation and fat class with greater consistency within and between abattoirs and predict whole carcase saleable meat yield, but further refinement is needed to improve prediction of saleable meat yield to the cut level. Based on the results of the experimental chapters, NIR could be used to identify *M. longissimus thoracis et lumborum* (LTL) with extremely high ultimate pH, shear force and intramuscular fat values. In addition, several questions that need consideration by the meat industry were identified. Further research is required to investigate the utility of alternative VIA outputs and to develop new calibration equations for saleable meat yield prediction. The relationship between LTL quality parameters and other muscles also requires further research before a meat quality-based carcase payment system based on an NIR scan of the LTL can be advocated.

10.1 Final conclusion

Research undertaken in the course of this project has contributed to the body of knowledge surrounding the effects of breed and gender on beef carcase quality and the ability of VIA to predict the yield of high-value cuts in beef carcases. Results of the current research have also provided further insights into genotype and gender effects on beef, lamb and venison meat quality, and have further characterized the relationships between NIR spectra and meat quality parameters for these three types of meat. Results of this research showed that NIR could be used to identify loin samples with extreme values for pH_{ult} (for beef and venison), IMF% (for lamb) and shear force (for venison, with some evidence for beef using a novel analysis method). It is still not clear whether measurements on loin are related to meat quality throughout the carcase. Further refinement to both VIA and NIR procedures are needed and should be driven by the requirements of the meat industry. There is a need to verify what the technologies can

realistically offer in terms of accuracy and precision before further consideration can be given to the use of VIA and NIR in commercial quality-based carcase evaluation systems.

11 Implications for industry

- VIA is able to classify carcases according to the EUROP grid in an automated manner but has scope for further refinement to predict cut yield. It is considered that the full potential of VIA has not yet been realized.

- The estimation of total carcase fat content continues to be a challenge, especially when assessment is largely based on subcutaneous fat cover. This will continue to hamper efforts to accurately determine saleable meat yield using VIA in its current form.

- Saleable meat yield prediction at the individual muscle or cut level is not yet possible at an acceptable level of accuracy, value-based marketing requires further developments in VIA to predict both yield and distribution of saleable meat in a carcase.

- Within the dairy crossbreds, *M. longissimus thoracis* from bulls was of poorer quality than steers and this effect was not due entirely to high ultimate pH in bulls. Due to a shortage of supply currently gripping the UK, bull beef may be utilized by markets generally accustomed to heifer or steer beef, this may have an impact on beef product consistency.

- Based on the current results and previously published research, NIR in its present form could be useful for identifying *M. longissimus thoracis et lumborum* with high ultimate pH, and extreme levels of toughness and could be used to improve the consistency of the product on offer to consumers.

- Industry needs to prescribe the requirements of a carcase evaluation system based on quality and address some of the challenges that will arise as outlined in Section 9.3.

- Identification of the issues surrounding the responsibility for “inferior” carcases and the consequences of this will help to determine the level of precision and accuracy required to move toward a value-based marketing system.

11.1 Next steps for research into prediction of meat quality

Research on meat quality encompasses a broad range of disciplines, ranging from muscle physiology to consumer psychology and microbiology to macroeconomics hence defining the next steps for meat quality research is difficult. Meat quality research is especially complex because there is a need to understand and quantify biological variation in meat animals, meat products and an equally important need to understand and quantify the variation in meat consumers. This is necessary in order to elucidate some of the causative factors affecting the meat quality as experienced by consumers and will enable the value chain to improve their processes. Whilst there is much work to be done in all these areas, industry in collaboration with meat science researchers should aim to better understand the interaction between consumers and meat products and determine how preference information can be relayed back along the value-chain.

11.2 The next steps for VIA and NIR research

The experiments and analyses undertaken in this thesis addressed several key areas several of which require more investigation. For VIA to be informative for carcase evaluation and breeding purposes and to play a role in a value-based marketing system, further development and analyses are required to:

- Develop robust prediction equations that can be applied at an industry level to identify carcases that have a higher yield of quality meat rather than to simply classify carcases according to the EUROP grid.
- Refine the prediction of SMY% to a level of resolution that is affordable and of significant value to the industry, preferably at the individual cut level.
- Determine the true potential of VIA with new prediction equations based on a gold standard such as CT scanning.

In terms of the prediction of meat quality, NIR has shown some promise for the loin, but further research is needed to:

- Establish whether measurements taken (or calibrations developed) on the loin can be used to determine quality of the other muscles.

- Develop calibration equations to predict meat quality across a wider range of genders and genotypes.

- Validate such equations on independent datasets.

- Evaluate new analysis techniques such as support vector machine regression, neural networks and genetic algorithms.

- Characterize the effects of different abattoirs and determine the capability of NIR (and other technologies such as hyperspectral imaging) to predict meat quality within and between abattoirs.

- Elucidate the biological systems underpinning the relationship between NIR spectra and meat quality attributes to identify causes and effects.

- Refine calibration equations according to the underlying biology and enable the use of more targeted and cost-effective instrumentation.

12 Bibliography

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13 Appendix

Table 13.1 Descriptive statistics for instrumental meat quality parameters of venison short-loin aged for 3 and 42 days as well as the combined (3d and 42d) dataset.

Parameter	dataset	*n*	Mean	SD	Range
Ultimate pH (pH_{ult})	3d	78	5.59	0.17	5.42-6.31
	43d	76	5.60	0.17	5.41-6.25
	all	154	5.60	0.17	5.41-6.31
Purge (%)	3d	78	2.96	1.05	0.00-5.53
	43d	76	4.87	1.46	2.00-8.85
	all	154	3.90	1.59	0.00-8.85
Expressed juice (EJ) (cm^2g^{-1})	3d	75	31.63	2.98	22.48-39.10
	43d	76	27.14	2.75	21.51-32.87
	all	151	29.37	3.64	21.51-39.10
Cooking loss (CL) (%)	3d	78	28.28	2.32	2.18-32.19
	43d	76	27.88	1.61	22.55-30.96
	all	154	28.08	2.01	21.76-32.19
Sarcomere length (SL) (µm)	3d	78	1.58	0.10	1.23-2.00
	43d	76	1.56	0.08	1.23-1.71
	all	154	1.57	0.09	1.23-2.00
Lightness (L*)	3d	78	35.31	2.37	29.66-41.12
	43d	76	37.38	1.79	30.73-40.09
	all	154	36.33	2.34	29.66-41.12
Redness (a*)	3d	78	11.71	1.60	6.86-14.86
	43d	76	12.31	1.39	7.7-14.81
	all	154	12.01	1.52	6.87-14.86
Yellowness (b*)	3d	78	2.95	0.84	0.92-4.75
	43d	76	3.27	0.71	1.39-4.58
	all	154	3.10	0.79	0.92-4.75
Warner-Bratzler peak shear force (WBSF) (kgF)	3d	78	7.64	1.81	3.90-13.07
	43d	76	5.14	1.27	3.13-9.52
	all	154	6.41	2.00	3.13-13.07
Initial yield force (IYF) (kgF)	3d	78	6.71	1.60	3.26-11.57
	43d	76	4.31	1.05	2.64-7.74
	all	154	5.53	1.81	2.64-11.57
PF – IYF (kg)	3d	78	0.93	0.49	0.15-2.58
	43d	76	0.83	0.48	0.15-2.36
	all	154	0.88	0.49	0.15-2.58
Work done (WD)	3d	78	2.30	0.51	1.06-3.48
	43d	76	1.67	0.39	0.99-2.93
	all	154	1.99	0.55	1.00-3.48

Table 13.2 Performance of NIR calibration equations showing the coefficient of determination (R^2) and standard error (SE) for calibration and full leave-one-out cross-validation for predicting instrumental meat quality parameters on venison short-loin within the 3d, 42d and the combined (3d and 42d) dataset.

				Calibration			Cross-validation		
Parameter	Dataset[a]	Pre-Treatment[b]	PC[c]	*n*[d]	R^2	SE	R^2	SE	RPD[e]
Ultimate pH (pH_{ult})	3d	MSC	4	78	62.0	0.11	55.6	0.12	1.42
	42d	MSC	4	76	78.7	0.08	73.2	0.09	1.89
	all	MSC	10	154	77.7	0.08	70.6	0.09	1.89
Purge (%)	3d	MSC	1	76	15.3	0.88	10.4	0.91	1.15
	42d	MSC	0	76	na[f]	na[f]	na[f]	na[f]	na[f]
	all	none	2	149	17.9	1.41	13.9	1.46	1.09
Expressed juice (EJ) (cm^2g^{-1})	3d	MSC	1	75	9.8	2.18	4.8	2.93	1.02
	42d	MSC	3	76	15.5	2.51	9.9	2.63	1.05
	all	MSC	0	154	na[f]	na[f]	na[f]	na[f]	na[f]
Cooking loss (CL) (%)	3d	MSC	4	77	23.9	2.02	13.9	2.17	1.07
	42d	MSC	6	75	45.5	1.19	21.0	1.45	1.11
	all	MSC	11	154	44.1	1.50	21.5	1.78	1.13
Sarcomere length (SL) (µm)	3d	none	2	78	38.4	0.09	20.1	0.09	1.11
	42d	none	2	76	35.4	0.06	26.7	0.07	1.14
	all	none	2	154	21.4	0.08	17.3	0.08	1.13
Lightness (L*)	3d	MSC	1	78	19.2	2.12	15.3	2.20	1.06
	42d	none	2	76	25.2	1.54	17.7	1.63	1.10
	all	none	5	154	35.4	1.87	30.2	1.96	1.19
Redness (a*)	3d	none	5	77	46.1	1.10	33.8	1.24	1.29
	42d	none	2	76	46.9	1.00	37.6	1.10	1.26
	all	none	4	154	46.5	1.11	42.0	1.16	1.31
Yellowness (b*)	3d	none	3	77	49.9	0.59	43.2	0.59	1.42
	42d	MSC	2	76	49.9	0.50	45.5	0.53	1.34
	all	none	3	154	49.0	0.56	45.7	0.59	1.34
Warner-Bratzler peak shear force (WBSF) (kgF)	3d	MSC	3	76	48.5	1.19	43.7	1.26	1.44
	42d	MSC	1	73	36.1	1.02	30.8	1.07	1.19
	all	none	6	152	47.4	1.39	39.6	1.50	1.33
Initial yield force (IYF) (kgF)	3d	MSC	2	76	35.5	1.17	28.4	1.25	1.28
	42d	none	0	76	na[f]	na[f]	na[f]	na[f]	na[f]
	all	none	6	151	46.6	1.26	40.3	1.34	1.35
WBSF – IYF (kg)	3d	none	3	78	42.1	0.37	36.2	0.40	1.23
	42d	MSC	2	76	60.7	0.30	57.2	0.32	1.50
	all	MSC	2	154	46.7	0.36	44.1	0.37	1.32
Work done (WD)	3d	none	4	76	50.3	0.34	43.8	0.36	1.42
	42d	none	3	75	32.0	0.32	25.2	0.34	1.15
	all	none	6	150	55.8	0.36	51.0	0.38	1.45

[a] dataset refers to samples aged for 3d or 42d before analysis, all is the combined dataset (3d and 42d aged samples).
[b] Pre-treatment indicates whether Multiplicative scatter correction (MSC) has been applied to the spectra prior to analysis.
[c] PC = number or principal components used in the regression.
[d] *n* = number of samples included in calibration and cross-validation phases.
[e] RPD = Ratio performance deviation (SD of the Y variable in the calibration dataset divided by the SE_{cv}).
[f] na = not available due to model failure.