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The use of computed tomography based predictors of meat quality in sheep breeding programmes

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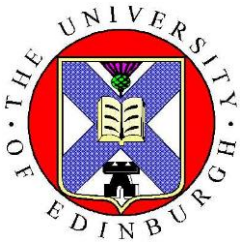
A Thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy



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Declaration

I hereby declare that this thesis is my own composition and that the research described in it is my own work, and all assistance has been duly acknowledged. The results presented herein have not previously been submitted for any other degree or qualification.

Neil Clelland

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I would like to dedicate this thesis to my family and my late father Hugh

"*Until a man has an objective he does not know what to do/learn. The choice of an objective mobilises the brain, it focuses the mental faculties upon achievement. It prevents desultory thought"*

Hugh Clelland (1943-1988)

Abstract

One of the main drivers influencing consumers in the purchasing of red meat is the level of visible fat, and this is particularly important in lamb, with lamb often being perceived as fatty. Consumer-driven preference for leaner meat, coupled with the meat processing industries preference for a reduction in carcass fat, increasing lean meat yield and reducing waste, have led to continued selection for lean growth and reduced fatness in several meat producing species The perception of lamb being fatty could be directly targeted in isolation by reducing overall fat levels, however there are related effects on meat (eating) quality, and the combined improvement and consistency of meat (eating) quality and the reduction of overall fatness is more complicated.

It is apparent that fat content plays a significant role in meat (eating) quality. Generally four major fat depots are recognised in animal carcasses, these are: subcutaneous (under the skin); internal organ associated; intermuscular (between muscles and surrounding muscle groups); and intramuscular (marbling, between muscle fibres), the latter generally regarded as having the greatest association with meat (eating) quality.

X-ray computed tomography (CT) can measure fat, muscle and bone *in vivo* in sheep and CT predictions of carcass composition have been used in commercial UK sheep breeding programmes over the last two decades. Together with ultrasound measures of fat and muscle depth in the loin region, CT measured carcass fat and muscle weights have contributed much to the success of breeding for leaner carcasses and increased lean meat yield. Recently it has also been considered that x-ray computed tomography provides the means to simultaneously estimate IMF and carcass fat *in vivo*.

Thus the aim of this project is to investigate the use of two and three-dimensional x-ray computed tomography techniques in the estimation of meat (eating) quality traits in sheep, and to further investigate the genetic basis of these traits and the possibility of their inclusion into current breeding programmes. The primary approach was the use of two-dimensional x-

ray computed tomography, determining the most accurate combination of variables to predict IMF and mechanical shear force in the loin. The prediction of mechanical shear force was poor with accuracies ranging from Adj R^2 0.03 – 0.14, however the prediction of IMF in the loin was more promising. CT predicted carcass fat weight accounted for a moderate amount of variation in IMF (R^2 =0.51). These accuracies were significantly improved upon by including other information from the CT scans (i.e. fat and muscle densities, Adj R^2 >0.65). Average muscle density in a single or multiple scans accounted for a moderate amount of the variation in IMF (Adj R^2 = 0.51-0.60), and again accuracies R^2 >0.65 were achieved, independent of CT-measured fat areas or predicted fat weights. Similar results were achieved with the use of three-dimensional CT scanning techniques (Adj R^2 0.51 – 0.71), however, there was a dramatically increased requirement for image analysis when compared to two-dimensional techniques, and the increase in accuracy was not significant. This suggests that the current method of two-dimensional image capture is sufficient in the estimation of IMF *in vivo* in sheep.

The prediction equations developed as part of this work were applied across divergent breed types (Texel, Scottish Blackface and Texel cross mule), to investigate the transferability of the prediction equations directly across to other breeds of sheep. As part of this study, the IMF levels across the breed types and sexes were also compared and found that IMF was significantly affected by breed type ($P<0.001$) with Scottish Blackface lambs having higher levels of IMF when compared to Texel cross mule lambs, and the lowest levels of IMF were in the purebred Texel lambs at the same liveweight or similar levels of carcass fatness. Sex also had a significant effect on IMF across breeds ($P<0.001$) with females having higher levels of IMF at similar levels of both carcass fat and liveweight, and within breed, females had significantly higher levels of IMF in both the purebred Texel and Scottish Blackface lambs, when compared at similar levels of carcass fat and liveweight ($P<0.05$). Using the models previously developed in purebred Texel to predict IMF in the Scottish Blackface and

Texel cross mule, accuracies were found to be R^2 = 0.57 – 0.64 and R^2 = 0.37 – 0.38 respectively. Providing evidence that the equations are transferable across to some breeds more successfully than others, however, given that there is currently no method of accurately estimating IMF *in vivo*, accuracies across to both breeds are acceptable.

The genetic parameter estimation was unsuccessful using the same research-derived dataset as previously employed in the study. However the ambition was always to investigate the genetic relationships between traits in a large industry dataset, exploiting the wealth of commercial CT information available. These investigations were considerably more successful, and among the first to present genetic parameters of novel CT-derived IMF estimates. The results found moderate heritability estimates of h^2 0.31 and 0.36 for the final selected prediction equations, with clear indications that one model not including CT predicted carcass fat or any other fat measures, was more independent of these measures and the two separate prediction methods were highly genetically correlated with each other (r_g = 0.89).

The results from this study show that not only is it possible to accurately estimate IMF levels in the loin of Texel sheep using CT scanning, but that, until breed specific predictions are developed, the methods developed in this study are transferable across some breed types. The results also show that CT predicted IMF is heritable, independent of overall fatness and has the potential to be included in current breeding programmes. These findings can now be used to develop breeding programmes which enable breeders to make the best use of CT scanning technology to improve carcass composition while maintaining or possibly improving aspects of meat (eating) quality.

Lay Abstract

One of the main drivers influencing consumers in the purchasing of red meat is the level of visible fat, and this is particularly important in lamb, as lamb often perceived as fatty. Consumer-driven preference for leaner meat coupled with the meat processing industries preference for a reduction in carcass fat, increasing lean meat yield and reducing waste, have led to continued selection for lean reduced fatness in several meat producing species. The perception of lamb being fatty could be directly targeted in isolation by reducing overall fat levels, however there are related effects to meat (eating) quality, and the combined improvement and consistency of meat (eating) quality and the reduction of overall fatness is more complicated.

It is apparent that fat content plays a significant role in meat (eating) quality. Generally four major fat depots are recognised in animal carcasses, these are: subcutaneous (under the skin); internal organ associated; intermuscular (between muscles and surrounding muscle groups); and intramuscular (marbling, between muscle fibres), the latter is generally regarded as having the greatest association with meat (eating) quality.

X-ray computed tomography (CT) can measure fat, muscle and bone in live sheep and CT predictions of carcass composition have been used in commercial UK sheep breeding programmes over the last two decades. Together with ultrasound measures of fat and muscle depth in the loin region, CT measured carcass fat and muscle weights have contributed much to the success of breeding for leaner carcasses and increased lean meat yield. Recently it has also been considered that CT provides the means to simultaneously estimate marbling and carcass fat *in vivo*.

Thus the aim of this project is to investigate the use of two and three-dimensional CT techniques in the estimation of meat (eating) quality traits in sheep, and to further investigate the genetic basis of these traits and the possibility of their inclusion into current breeding

programmes. The primary approach was the use of two-dimensional CT, determining the most accurate combination of variables to predict marbling and tenderness in the loin. The prediction of tenderness was poor with accuracies ranging from 3% to 14% (100% accuracy being the best), however the prediction of marbling in the loin was more promising. Simple CT variables predicted marbling with around 51% accuracy. These accuracies were significantly improved upon by including other information from the CT scans, which increased the accuracy to more than 65%. Similar results were achieved with the use of three-dimensional CT scanning techniques (51% – 71% accuracy), however, there was a dramatically increased requirement for image analysis when compared to two-dimensional techniques, and the increase in accuracy was not significant. This suggests that the current method of two-dimensional image capture is sufficient in the estimation of marbling in live sheep.

The prediction equations developed as part of this work were applied across very different breeds (Texel, Scottish Blackface and Texel cross mule), to investigate the transferability of the prediction equations directly across to other breeds of sheep. As part of this study, the marbling across the breed types and sexes were also compared and found that marbling was significantly different across breeds with Scottish Blackface lambs having higher levels of marbling when compared to Texel cross mule lambs, and the lowest levels of marbling were in the purebred Texel. Sex also had a significant effect on marbling in the different breeds with females having higher levels of marbling. Within the same breed, females had significantly higher levels of marbling in both the purebred Texel and Scottish Blackface lambs. Using the models previously developed in purebred Texel to predict marbling in the Scottish Blackface and Texel cross mule, accuracies were found to be 57% – 64% in Scottish Blackface and 37% – 38% in Texel cross mule. Providing evidence that the equations are transferable across to some breeds more successfully than others, however, given that there

is currently no method of accurately estimating marbling in live sheep, accuracies across to both breeds are acceptable.

The genetic analyses found that marbling predicted by CT may be passed on through genetics, with clear indications that one model not including overall fat or any other fat measures, was more independent of these measures, and the two separate prediction methods were found to be genetically the same.

The results from this study show that not only is it possible to accurately estimate marbling in the loin of Texel sheep using CT, but also that the methods developed in this study are transferable across some breed types. The results also show that marbling predicted by CT is heritable, independent of overall fatness and has the potential to be included in current breeding programmes. These findings can now be used to develop breeding programmes which enable breeders to make the best use of modern technology to improve carcass quality while maintaining or possibly improving aspects of meat (eating) quality.

Publications

Peer reviewed publications

Clelland, N. Bunger, L. McLean, K.A. Conington, J. Maltin, C. Knott, S and Lambe, N.R. 2014. Prediction of intramuscular fat levels in Texel lamb loins using X-ray computed tomography scanning. Meat Science 98, 263-271. (Based on Chapter 2)

Conference proceedings and presentations

Clelland, N. Lambe, N.R. Knott, S.A. Conington, J and Bunger, L. 2013. Investigating image analysis techniques for predicting intramuscular fat percentage from computed tomography reference scanning (two-dimensional information) in Texel lambs. Proceedings of the British Society of Animal Science, University of Nottingham April 2013, 142

Clelland, N. Bunger, L. McLean, K.A. Knott, S. and Lambe, N.R. 2013. Prediction of intramuscular fat in Texel lamb loins using different x-ray computed tomography (CT) scanning techniques. Proceedings of the Farm Animal Imaging (FAIM) conference, Kaposvar, Hungary, 53-56

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Clelland, N. Bunger, L. Knott, S. Menezes, A.M and Lambe, N.R. 2014. Predicting intramuscular fat content in the loins of divergent sheep breeds using x-ray computed tomography. Proceedings of the Farm Animal Imaging (FAIM) conference, Copenhagen, Denmark, 48-51

Associated authorship

Bunger, L. Clelland, N. Moore, K. McLean, K.A. Kongsro, J and Lambe, N.R. 2014. Integrating computed tomography (CT) into commercial sheep breeding in the UK: cost and value. Proceedings of the Farm Animal Imaging conference, Copenhagen, Denmark, 22-27

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List of Abbreviations

Abbreviation	Explanation
AHDB	Agriculture and horticulture development board
ANOVA	Analysis of variance
AOAC	Association of analytical communities
BioSS	Bio-informatics and statistics Scotland
Chem_IMF	Chemically extracted intramuscular fat
CT	X-ray computed tomography
CT_Age	Age at X-ray computed tomography scanning
CTLWT	Live weight at CT scanning
CTMD	Computed tomography muscle density
EBLEX	English Beef and Lamb Executive
EU	European Union
FD	Fat density
FSD	Standard deviation of fat density
f_vol	Fat tissue volume
HU	Hounsfield unit
IMF	Intramuscular Fat
ISC	Ischium
ISCFA	Fat area in the ischium region
ISCFD	Average fat density in the ischium region
ISCFSD	Standard deviation of fat density in the ischium region
ISCMA	Muscle area in the ischium region
ISCMD	Average muscle density in the ischium region
ISCMSD	Standard deviation of muscle density in the ischium region
ISCSTD	Average soft tissue density in the ischium region
ISCSTSD	Standard deviation of soft tissue density in the ischium region
KgF	Kilogrammes of force
LV5	5^{th} lumbar vertebra
LV5FA	Fat area in the 5^{th} lumbar vertebra region
LV5FD	Average fat density in the 5^{th} lumbar vertebra region
LV5FSD	Standard deviation of fat density in the 5^{th} lumbar vertebra region
LV5MA	Muscle area in the 5^{th} lumbar vertebra region
LV5MD	Average muscle density in the 5^{th} lumbar vertebra region
LV5MSD	Standard deviation of muscle density in the 5^{th} lumbar vertebra region
LV5STD	Average soft tissue density in the 5^{th} lumbar vertebra region
LV5STSD	Standard deviation of soft tissue density in the 5^{th} lumbar vertebra region
LW	Live weight
MD	Muscle density
MEQ	Meat eating quality
MSD	Standard deviation of muscle density
MQ	Meat quality
OLS	Ordinary least squares
PLS	Partial least squares

Abbreviation	Explanation
Pr_Cfat	Computed tomography predicted carcass fat
Pr_IMF	X-ray computed tomography predicted intramuscular fat
RMSE	Residual mean square error
RMSEP	Residual mean square error of prediction
ROI	Region of interest
SBF	Scottish blackface sheep
SCTS	Spiral computed tomography scanning
SD	Standard deviation
ShF	Mechanical shear force
SL_Age	Age at slaughter
SRUC	Scotland's rural college
STAR	Sheep tomogram analysis routines
STD	Soft tissue density
STSD	Standard deviation of soft tissue density
Tex	Texel sheep
TexX	Texel crossed with Mule sheep
TV8	8^{th} thoracic vertebra
TV8FA	Fat area in the 8^{th} thoracic vertebra
TV8FD	Average fat density in the 8^{th} thoracic vertebra region
TV8FSD	Standard deviation of fat density in the 8^{th} thoracic vertebra region
TV8MA	Muscle area in the 8^{th} thoracic vertebra
TV8MD	Average muscle density in the 8^{th} thoracic vertebra
TV8MSD	Standard deviation of muscle density in the 8tht thoracic vertebra region
TV8STD	Average soft tissue density in the 8^{th} thoracic vertebra region
TV8STSD	Standard deviation of soft tissue density in the 8^{th} thoracic vertebra region
UK	United Kingdom
US	Ultrasound
w_fd	Weighted average fat density
w_fsd	Weighted standard deviation of fat density
w_md	Weighted average muscle density
w_msd	Weighted standard deviation of muscle density
w_std	Weighted average soft tissue density
w_stsd	Weighted standard deviation of soft tissue density
m_vol	Muscle tissue volume

Chapter 1: General Introduction

1.1 Background

One of the main drivers influencing the decisions made by consumers at point of purchase with regard to red meat, as highlighted by the English beef and lamb executive (EBLEX) report (2010), is the level of visible fat associated with lamb. This report highlights that lamb is often perceived as fatty by the consumer. The perception of lamb being fatty could be directly targeted in isolation, however the combined improvement and consistency of meat quality (MQ) and associated meat eating quality (MEQ) characteristics alongside the reduction of overall fatness is more complicated and should be considered in future breeding programmes.

The main quality attributes of meat can be determined in different ways. Measurements of MQ usually describe technological or mechanical factors, such as shear force (SF), colour, or chemical and toxicological information (e.g. fatty acid profiles, chemically extracted fat content, levels of bacteria, pH etc.), whilst MEQ describes quality attributes concerned with the consumption of fresh meat products relating to organoleptic traits, considering properties such as flavour, tenderness and juiciness. This can only be directly evaluated by a taste panel, but there are methods to predict it, which all need to be calibrated against taste panel results in the first place.

In different livestock species, MEQ traits such as flavour, tenderness and juiciness are known to be linked to fat levels (Brewer et al., 2001; Fernandez et al., 1999; Fortin et al., 2005; Killinger et al., 2004; Savell and Cross, 1988; Smith and Carpenter, 1976).

It is apparent that the fat content of meat plays a significant role in the acceptability of major MEQ attributes concerning the consumer and for many decades the influence of fat content on the eating quality of meat has been debated. Generally four major fat depots are recognised in animal carcasses: subcutaneous (under the skin); internal organ associated

(visceral fat, also known as intra-abdominal or organ fat; composed of several adipose depots including mesenteric, epididymal white adipose tissue and perirenal fat); intermuscular (between muscles and surrounding muscle groups); and intramuscular fat (IMF, interspersed within skeletal muscle and between muscle fibres), the latter having the greatest association with MEQ (Brewer et al., 2001; Fernandez et al., 1999; Fortin et al., 2005; Killinger et al., 2004; Savell and Cross, 1988; Smith and Carpenter, 1976).

Consumer-driven preference for leaner meat, coupled with the meat processing industries preference for a reduction in carcass fat, increasing lean meat yield and reducing waste, have led to continued selection for lean growth and reduced fatness in several meat producing species (Simm and Dingwall, 1989; Sonesson et al., 1998). However, IMF and back fat thickness are genetically positively correlated in these meat producing species (pigs; r_g 0.31), which has resulted in a decrease in IMF content in pigs through genetic selection for decreased back fat which has in turn had a negative effect on the palatability of fresh pork (Sonesson et al., 1998). The genetic correlations between meat quality traits and carcass composition traits have also been investigated in sheep, Lorentzen and Vangen (2012) reporting a moderately high genetic correlation between IMF and dissected fat (kg) (r_g 0.62) and a similar situation about the impact of selection for leaner carcasses to that of the pork industry has been reported to be emerging in the Australian sheep industry (Pannier et al., 2014a).

Given the genetic relationship between IMF and carcass fat and the possible impact on MEQ previously mentioned, it has been recognised that there is a need to have independent estimates for carcass fat and IMF enabling selection against this positive correlation. Any such divergent selection would not be possible, or at the very least difficult, if the genetic correlation was as a result of pleiotropic genes or tight gene linkage. However, there is

evidence that different fat depots are at least partially controlled by different genes in both mice and pigs (Bunger and Hill, 2005; Gerbens et al., 1999).

X-ray computed tomography (CT) can measure fat, muscle and bone *in vivo* in sheep and CT predictions of carcass composition have been used in commercial UK sheep breeding programmes over the last two decades (Bunger et al., 2011). Together with ultrasound measures of fat and muscle depth in the loin region, CT measured carcass fat and muscle weights have contributed much to the success of breeding for leaner carcasses and increased lean meat yield (Jopson et al., 2004; Lewis and Simm, 2002; Moore et al., 2011). However, previous research has not only demonstrated that CT can estimate carcass fat, but it also provides measurements of the average CT muscle density (CT MD), which is a good predictor of IMF. Strong negative correlations were found between IMF and CT MD in different sheep breeds (Karamichou et al., 2006; Macfarlane et al., 2005; Navajas et al., 2008; Young et al., 2001). Taste panel scores for MEQ traits such as flavour, juiciness and overall palatability were also shown to have strong negative genetic and phenotypic correlations with CT MD (Karamichou et al., 2006). Advances in CT technology have provided the availability to perform spiral CT scanning, improving the quality and amount of detailed images available through CT, in contrast with earlier 'step and shoot' techniques which involved taking a 'slice' of an area of interest and then moving on to the next area of interest. The use of spiral CT, which is able to capture detailed three-dimensional information, may allow further advances in predicting aspects of meat quality. CT provides the means to quantify simultaneously and independently both IMF and carcass fat *in vivo* enabling these estimates to be exploited in selection programmes simultaneously choosing breeding animals with low carcass fat alongside optimum levels of IMF.

1.2 Structure of sheep farming in the UK

In 2013 approximately one quarter of the sheep population in the European Union (EU) was to be found in the United Kingdom (UK) around 22.5 million sheep of approximately 88.5 million sheep in the EU. Of this around 14.8 million were breeding ewes. The major ram breeds used in the UK are the terminal sire breeds, Texel, Suffolk and Charollais, accounting for around 50% of the rams used in the UK with around 20% of the breeding ewes are mated to Texel rams (Pollott, 2012).

There is huge diversity in the topography and climate of sheep farming in the UK, this diversity in environment and landscape dictates different systems, with specific breed types. Although there is diversity in environment, landscape and as a result, farming systems within the UK, sheep production is integrated through a unique stratified system (see Figure 1.1). The stratification is made up of three tiers: hill; upland and lowland.

Top down, the structure of this stratified system begins with the farming of hill specific breeds maintained as pure breeding flocks, suited to the harsh environment of hill and mountain landscapes. Breeding hill ewes are then drafted onto upland farms following three or four lamb crops, where they are then crossed with 'Longwool' breeds. Replacement ewes are then chosen from these Longwool crossbred lambs, drafted to lowland farms and crossed with the terminal sire breeds mentioned previously for the production of prime lamb for meat. This system focuses entirely on the production of prime lamb for meat as this is the major contributor to economic returns for UK sheep farmers.

Given this emphasis on meat production, sheep producers have concentrated on the improvement of lean meat yield and quality (the definition of which differs across the production chain, and is discussed in detail later in this chapter) in the carcass, through the use of genetic selection.

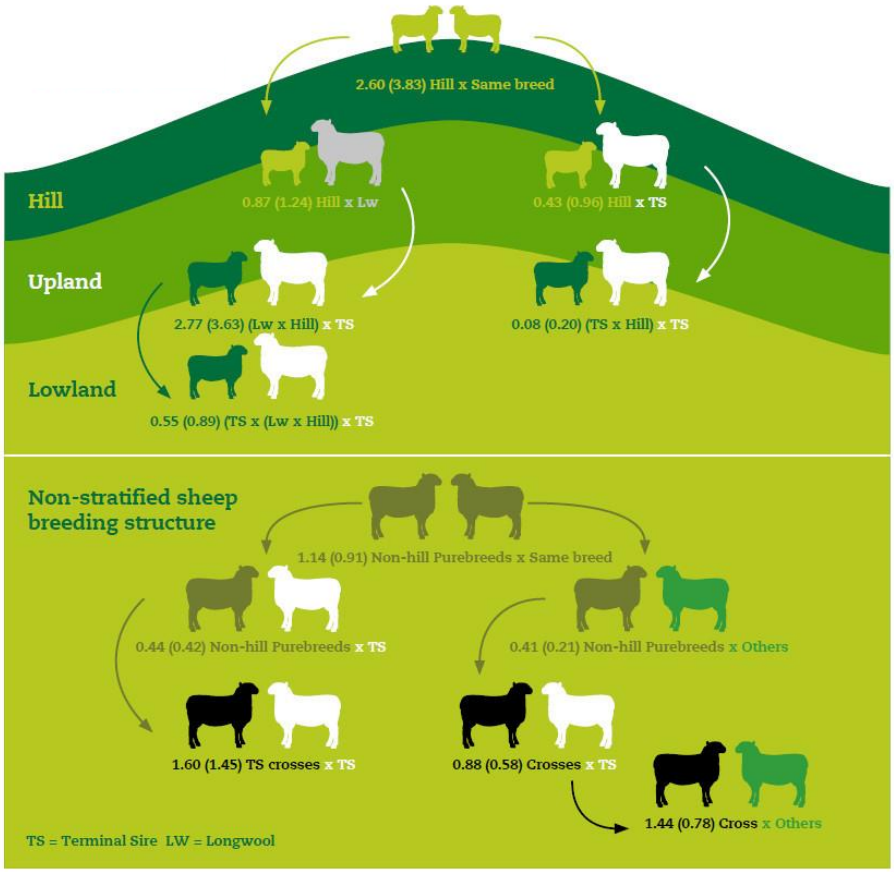


Figure 1.1 Diagrammatic representation of the stratified UK sheep breeding structure (top) and non-stratified sheep breeding structure (bottom) with 2003 ewe numbers shown in brackets (million) from Pollott (2012)

1.3 Improvement through genetic selection

Improvement through genetic selection relies on genetic variation between animals in the traits of interest. Modern domesticated sheep breeds are a direct result of selective breeding, and historically this was driven mainly by the visual appraisal of desirable traits. This historical selection was carried out in the absence of any real knowledge of genetics. However these principles of Mendelian inheritance underpin the selection practices.

Classical genetics and the development of modern quantitative genetics simply extended Mendelian principles of inheritance to the wider 'population' and instead of visual appraisal,

included the ‘measurement’ of traits of interest (phenotypes), resulting in the development of population and quantitative genetics (Falconer and Mackay, 1996).

The development of quantitative genetics has now made it possible to select for and improve traits of interest through breed improvement schemes, and as a result improve breeds through increased efficiency of production. These genetic improvements in efficiency of production are particularly valuable to the livestock industries involved as, when selection is continuous, genetic improvement is both permanent and cumulative (Simm, 1998).

Generally, improved breeds of livestock produce protein, fibre or other products more efficiently, of relative higher quality and are ultimately better matched to modern market needs than their antecedents. For example, genetic improvement in pigs and poultry, broadly in the efficiency of the production of meat, has changed the products from these species from being luxury foods to the cheapest meats available.

Quantitative genetics relies on the knowledge about the pedigree of individuals in a population alongside phenotypic performance data related to traits of interest. With this detailed information, we can make useful inferences about the inheritance of any traits of interest. We can infer that genes make an important contribution to the phenotypic variance in the trait of interest in individuals that share a similar pedigree and are phenotypically similar, and therefore share lots of alleles.

The amount of phenotypic variance (V_P) that is due to genetic differences (V_G) among individuals can be estimated using the pedigree and performance related data, however, these genetic differences are made up of additive genetic (V_A), dominance (V_D) and epistatic (V_I) sources of variance. In non-experimental settings V_D and V_I are difficult to estimate and as such, animal breeders tend to focus on estimating V_A by partitioning V_P in to two parts, V_A +

V_R where V_R is the residual variance and is normally interpreted as an environment effect (Falconer and Mackay, 1996).

The proportion of V_P explained by V_A is then defined as the narrow sense heritability of a trait (h^2). The heritability of a trait is the proportion of superiority of parents (i.e. the proportion of the selection differential) which is passed on to progeny.

However, in livestock production systems, increased efficiency or improved production relies on several different traits. For example, in meat producing species, economic returns are based on multiple factors, such as, litter size, growth characteristics, and the weight of a carcass, fatness, lean meat yield (conformation) and feed efficiency. And as a result of this combination of factors, animals in breeding programmes are usually selected on a combination of traits.

The common method of selection for multiple traits of interest is to use a selection index, in which an overall score reflecting genetic merit in the combination of traits of interest with an optimum economic balance is calculated. In order to calculate such an index it is necessary not only to know the heritability for the trait of interest but also the relationship, their genetic correlations, between the traits included in the index. These relationships may be estimated by calculating how much of the phenotypic covariance (COV_P) is explained by additive genetic effects (COV_A). This additive genetic covariance is used to derive genetic correlations (r_g) and is expected, through either the tendency of alleles that are located close together on the chromosome to be inherited together during meiosis, termed 'genetic linkage', or when one gene influences multiple traits, termed 'pleiotropy'.

Breeding schemes have been adopted broadly across economically important livestock species and breeds, which involve the recording and sharing of pedigree and phenotypic performance related data, allowing breeders to select for important traits related to

economical, production and welfare gains, tailored to any specific species, breed or market demand.

1.4 Meat quality

Meat is a major food item worldwide and has traditionally held high status as a source of protein in Western food culture. Most recipes and meals are named after the meat utilised in the recipe, and as such the type or form of the meat used in a recipe is often seen to be the central part of the meal (Aaslyng, 2009; Douglas and Nicod, 1974; Gvion-Rosenberg, 1990; Holm and Mohl, 2000).

Such is the importance of meat in Western food culture and as part of a meal it has even translated to the Western vegetarian meal structure. In vegetarian dishes the main component of the dish is often used as if it were meat, or presented in such a way as to resemble meat (Gvion-Rosenberg, 1990; Holm and Mohl, 2000).

The definition and understanding of meat quality is complex and considers many factors, both intrinsic factors such as, appearance, colour, flavour, juiciness etc., and extrinsic factors such as, price, brand name and quality assurance schemes, country of origin and production processes (Issanchou, 1996; Oude Ophuis and Van Trijp, 1995; Steenkamp, 1989).

These attributes of meat quality also depend on the intended use of the meat and as such, and as mentioned in section 1.1, quality can be broadly defined as ‘technological quality’, describing meat for further processing, or as ‘fresh meat eating quality’, describing meat for consumption.

In more detail, fresh meat eating quality includes organoleptic traits (traits registered with our senses) such as appearance, tenderness, juiciness and flavour. These meat eating quality

indicators overlap to some degree with technological quality indicators, but some differences also exist (Aaslyng, 2002; Becker, 2009).

Quality, in both terms, ultimately can mean different things to different people involved in the production cycle, varying between producer, processor, retailer and consumer, and also within different market groups. (Becker, 2009; Oude Ophuis and Van Trijp, 1995).

1.5 Meat eating quality

Eating meat for pleasure is the simplest form of associating factors of MEQ. For products such as steaks, chops and roasts, three sensory attributes of major importance are; tenderness, juiciness and flavour (Aaslyng et al., 2007; Bryhni et al., 2003; Maltin et al., 2003; Miller et al., 2001; Resurreccion, 2004).

The ranking of importance of these factors varies geographically as reported by Jeremiah *et al* (1994) and Aaslyng *et al* (2007). Also the relative ranking of importance varies depending on the associated satisfaction of the other organoleptic traits (Aaslyng et al., 2007; Huffman et al., 1996; Killinger et al., 2004). For example, when meat is tender and satisfies this factor fully, juiciness and flavour have a larger impact on consumer preference. But when the meat is tough, tenderness becomes the most important factor (Miller et al., 2001).

However, these factors can only be assessed by the consumer on cooked meat. Therefore, when the consumer is buying meat, it is only possible to make any estimation of the eating quality of that meat from other quality attributes, such as the appearance of the raw meat. Therefore, the appearance of raw meat is very important in the consumer's behaviour at point of purchase.

1.5.1 Appearance

Leanness, intramuscular fat content (marbling) and colour rank highly in the consumers perception of fresh meat quality (beef, pork and chicken) in a study carried out by Glitsch (2000) across 6 European countries (Germany, Ireland, Italy, Spain, Sweden and the UK). In the purchase of lamb meat both colour and subcutaneous fat are the two main drivers of acceptability in appearance at the point of purchase. The consumer associates the colour of meat with freshness, while a visible layer of subcutaneous fat is associated with possible adverse health implications for the consumer and as a result low fat cover is preferred by most consumers in the UK (Aaslyng, 2002; Aaslyng, 2009; Allen, 2010) . However it is acknowledged that these preferred levels of fatness (and colour) vary depending on the production process (cured hams, milk fed lamb, veal etc.) and the country, or indeed continent, in which the meat is purchased. It should also be noted that there are differences between varying demographics and consumer groups within country (Font-i-Furnols and Guerrero, 2014).

This preference for reduced visible fatness can also be interpreted by the consumer through visible IMF levels in some species. These visible levels of IMF in beef have influenced consumers at point of purchase, with consumers preferring lower levels of IMF at point of purchase. However, the same consumers preferred higher levels of IMF when consuming the products (Jeremiah et al., 1992; Risvik, 1994) similar findings of the preference for reduced visible fat levels were also presented in a broad range of meat producing species, including pork and lamb (Font-i-Furnols and Guerrero, 2014).

1.5.2 Tenderness

For the consumer tenderness is often described as the most important factor for high eating quality in meat, and it has been shown that the level of tenderness is crucial to consumer

acceptability, so much so that consumers are willing to pay more for increased levels of tenderness (Aaslyng, 2009; Boleman et al., 1997; Huffman et al., 1996; Kamruzzaman et al., 2013; Maltin et al., 2003; Naganathan et al., 2008; Risvik, 1994). It is possible to measure tenderness through consumer or trained taste panels, and also using mechanical shear force tests performed in laboratory conditions. There are several methods of shear force tests which may be carried out, such as the Volodkevich (Volodkevich, 1938), Warner-Bratzler (Bratzler, 1949) and the meat industry research institute of New Zealand (MIRINZ) method (Macfarlane and Marer, 1966). All these methods are applied to samples of cooked meat across the muscle fibre axis, samples are cooked to a pre-determined temperature to reduce any effect of cooking temperature on tenderness, and sampled at standardised dimensions. Cooking temperatures and sample sizes vary across laboratories and methods. However the overall principle remains the same, the force required to shear or 'bite' the sample is measured, with higher peak force values indicating tougher samples. Several samples may be taken form certain cuts and averaged in order to be more representative of the perceived tenderness from that particular cut. These methods are applied in order to reduce the cost of trained sensory or consumer taste panels. This, of course, relies on the correlation between trained sensory panels and shear force results and several studies have investigated these relationships across several different methods and species with correlations ranging from r = -0.36 (Lambe et al., 2011) in lamb using the Volodkevich method to r = -0.71 (Safari et al., 2001) also in lamb using the Warner-Bratzler method. Another study in beef carried out by Ross *et al* (2009) involving several methods and ageing times reported similar correlations (r = -0.47 to -0.60). These relationships are important to understand when using an objective measure (shear force) to quantify a subjective trait (tenderness in sensory panels). It is also important to understand that consumers are able to easily distinguish between very tender and very tough muscles. However, for the consumer, variation of tenderness within these two extremes is more difficult to determine (Maltin et al., 2003; Siversten et al., 2002). The method of objectively determining perceived tenderness may possibly be improved upon by

plotting and analysing the entire ‘tenderness curve’ of a sample during shear force testing, and relating this to the organoleptic trait.

There are different ways to achieve or increase the tenderness of meat, both *in vivo* and *post-mortem*. The two most important of these are the degree of intramuscular fat (*in vivo*) and conditioning or ageing of the meat (*post-mortem*) (Brewer et al., 2001; Fortin et al., 2005; Savell and Cross, 1988; Smith and Carpenter, 1976; Therkildsen, 1999). Smith and Carpenter (1976) summarised the effect of IMF on tenderness and juiciness, based on the collective or independent effects of the following theories:

Bite theory: suggests that within a bite size portion of cooked meat, IMF content decreases the mass per unit volume, and as fat is less resistant to shear force than muscle fibres, this decrease in bulk density results in a real or apparent increase in tenderness.

Lubrication theory: is that the presence of IMF in and around the muscle fibres, retain moisture and lubricate the fibres and fibrils increasing the perceived juiciness and tenderness, also highlighting the close association of tenderness and juiciness.

Insurance/Insulating theory: considers that increased levels of IMF insure and insulate muscle fibres from the use of high temperature, dry heat cooking methods and increasing degrees of “doneness”, providing some insurance that cuts of meat that are cooked either too long or too rapidly will still be tender and juicy.

The insurance/insulating theory also highlights the matching of certain cuts of red meat to levels of doneness, particularly in beef, hence cuts with lower levels of IMF are recommended to be eaten at lower levels of doneness and fattier cuts are more suitable for higher levels of doneness.

There are conflicting results about the influence of the levels of fat on tenderness, as it has been shown in some studies to increase tenderness in different species including pigs (Brewer et al., 2001; D'Souza and Mullan, 2002; Fernandez et al., 1999), beef (Gwartney et al., 1996) and lamb (Smith et al., 1976). However, it has also been shown to have little or no influence on tenderness in pork (Rincker et al., 2007). Although there are some conflicting results on the relationship between IMF and tenderness in meat producing species, it is widely regarded that IMF content has a positive effect on tenderness. The variation in IMF content seems to be the limiting factor in its relationship to tenderness, with extreme levels of IMF (high or low) having the most effect on tenderness.

1.5.3 Flavour

As mentioned previously, flavour is also a very important attribute in relation to the eating quality of meat. In the overall palatability of meat, flavour was regarded as being equally influential, alongside tenderness, in determining overall liking in several cuts during US customer satisfaction surveys involving beef (Calkins and Hodgen, 2007). Preferred flavours in meat are very important, but equally it is important to avoid off flavours.

There are literally hundreds of compounds that may contribute to the flavour and aroma of meat, as complex interactions between these compounds influence flavour. The flavour of meat can be influenced by oxidation, lipid content, diet, and pH (Calkins and Hodgen, 2007).

The fried flavour of meat is generated through the heat treatment during the cooking process as flavour and aroma compounds are formed by several pathways including lipid oxidation and non-enzymatic browning also known as the Maillard reaction (Maillard, 1912). The reactions from these two systems result in a large number of volatile compounds, and the thermal degradation of other compounds, such as thiamine, contribute to the flavour of meat (Calkins and Hodgen, 2007; Mottram, 1998).

It has been demonstrated that an increase in IMF corresponds with an increase in the acceptability of fried flavour of beef and pork (Brewer et al., 2001; Killinger et al., 2004).

The effect that IMF has on flavour, could be due to improved flavour release during cooking, not only increased flavour development in the meat, as fat acts as a storage depot for flavour and odour compounds which are released on heating. It is also widely regarded that fat contributes more to species-specific flavours and odours in meat, with lean tissue containing the precursors for the meaty flavour characteristics of all meats (Mottram, 1998; Savell and Cross, 1988).

Even though flavour is an important factor in MEQ and consumer preference, the consumer's willingness to pay for improved flavour attributes has not been investigated as fully as that of tenderness attributes in meat.

1.5.4 Juiciness

Juiciness is seen as another important MEQ factor. The importance of juiciness for MEQ depends firstly on the composition of the meal, and the cooking processes involved. For example, a grilled steak, cooked at high temperature for a short period, will need to be juicier than smaller pieces of diced meat in a stew, cooked at a moderate temperature for a longer period.

The main determining factor of the juiciness of meat is the final core temperature. Increases in the final core temperature will see an increase in cooking loss and therefore a decrease in juiciness (Aaslyng et al., 2007; Sheard et al., 1998). An increased amount of IMF has been shown to increase or maintain the juiciness of the meat when fried to a high final core temperature. At a lower final core temperature, IMF has no effect on juiciness (Aaslyng, 2009). These results agree with Smith and Carpenters (1976) insurance/insulation and

lubrication theories, highlighting again the close association of perceived juiciness and tenderness of meat in relation to IMF content.

In consideration of the effect of IMF levels in meat and the resulting perceived juiciness from the consumers' point of view, an important factor must be the education of the consumers' on cooking times and techniques, and to educate the consumer in optimum final core temperature while cooking the meat to obtain the best results for juiciness, however this would only be achievable if a consistent product is supplied in terms of IMF levels.

1.6 X-Ray Computed Tomography

X-ray computed tomography (CT) was initially developed for use in human medicine and introduced into clinical practice in 1972. CT was the first of the modern slice-imaging modalities, reconstructing images mathematically from measured data allowing the display and archiving of images. CT has since developed with the introduction of spiral scanning (SCTS) providing advances from single-slice scanning allowing true volume imaging capabilities realised by SCTS.

The theory on which CT is based can be traced back to the early 20th Century, and some of the ideas on which it was based, mainly that the density and distribution of a material can be calculated if the attenuation value of any number of lines passing through the same material can be measured, was first explored by the Austrian mathematician, Johann Radon (1917).

The first experiments on medical applications for reconstructive tomography were carried out by the physicist A.M. Cormack in the late fifties into the early sixties. Without any knowledge of previous studies, based on transmission measurements, Cormack was able to develop a method of calculating the absorption of x-ray radiation and distributions in the human body (Cormack, 1963). Although Cormack's work is now regarded as essential to the

development of CT, there were many other researchers also investigating the development of transmission and reconstruction techniques in the sixties and seventies (Gordon et al., 1970; Kuhl and Edwards, 1963; Oldendorf, 1961; Shepp and Logan, 1974).

The British engineer Godfrey Hounsfield, also unaware of earlier research, achieved the first successful practical implementation of the theory in 1972, and is now broadly recognised as the inventor of modern CT (Hounsfield, 1973). Hounsfield and Cormack's contributions earned them the Nobel Prize in medicine in 1979.

1.6.1 Basic Principles of computed tomography

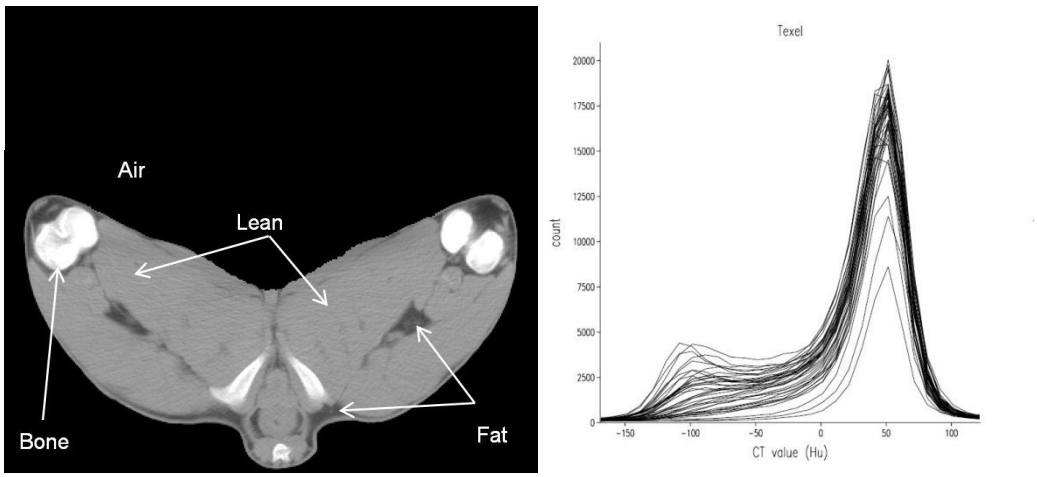
The basic principles of CT are based on X-ray attenuation through an object. An X-ray source and detector rotate 360^{O} around an object and from this X-ray images are generated as different tissues generate different degrees of X-ray attenuation, providing information on the density of these tissues (Harvey and Blomley, 2003; Kalender, 2005). This information can then be reconstructed to form a CT image (tomogram) of the subject internally and externally.

Air is transparent to X-rays and as a result appears black on the reconstructed CT image, high density elements of the image will appear white and the gradients of grey between these values indicate density values related to soft tissues within the subject, such as fat and lean (Figure 1.2a). These values of attenuation are quantified in Hounsfield units (HU), calibrated using the baseline value of distilled water (HU = 0). Images can then be analysed according to the HU value of each pixel within that image, and histograms or profiles of the distribution can be visualised and analysed (Figure 1.2b).

To enable more accurate estimation of the distribution of different tissues (e.g. fat, muscle, bone) within an image two approaches may be used; firstly *a priori* knowledge of CT values

and determined thresholds may be used to partition distributions within tissue densities (Kalender, 2005), this method assumes no differences in HU thresholds for the same tissue within populations or across genotypes, or between scanner types. Secondly and in particular in livestock species, the calibration of histograms against a known reference method such as manual dissection may be carried out to determine threshold levels (Jones et al., 2002; Lambe et al., 2003; Lambe et al., 2006; Navajas et al., 2007). This method ensures that HU values are calibrated against real data which has been extracted from the species or genotype being modelled and using the equipment which will be used in future estimations (scanner type).

Each pixel can then be allocated to fat, lean or bone as the value determined will represent the average density within the area covered by the pixel. A pixel may border tissue types, for example, the density value of a pixel bordering fat and lean tissue will be determined by the average of these two tissues, these 'mixed pixels' contribute to the partial volume effect also known as partial volume averaging (Schwarz, 2011). The allocation of pixel number associated to each HU value will reduce the partial volume effect and the further allocation of density within the pixel may reduce this further (Font-i-Furnols et al., 2013)

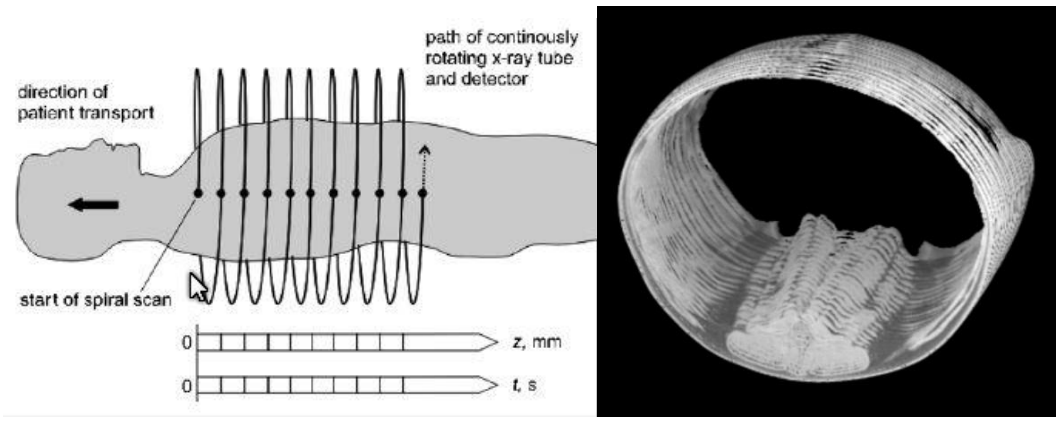


a) **b)**

Figure 1.2 a) CT tomogram image through the upper hind legs of a lamb, detailing tissue grayscales representing corresponding density values **b)** Distribution of HU values of pixels from CT scans taken on a number of Texel sheep, the first peak identified as fat tissue (left) and the second peak identified as lean tissue (right)

Further developments in CT technology saw the introduction of spiral CT scanning (SCTS) in 1989 (Kalender et al., 1990; Kalender, 1994; Kalender, 1999; Kalender and Polacin, 1991) and following this development, scanners can be used to generate three-dimensional images, enabling not only the measurement of density related to X-ray attenuation, but also as a result of the reconstructive three-dimensional ability, very precise measures of the volume of tissues.

This is made possible by the continuous scanning along a subject's longitudinal axis (*z*-axis) producing a contiguous scan image. To achieve this contiguous scan, the table on which the subject is positioned is moved at a precise speed of one slice thickness per rotation through the gantry (Figure 1.3a), from this, 3 dimensional reconstructions of the subject are possible (Figure 1.3b)



a) **b)**

Figure 1.3 a) Spiral CT focus trajectory from Kalender (2006) **b)** *in-vivo* segmented 3D image of lamb loin

It should be noted that currently CT is being considered by the EU as a reference method to replace manual dissection in pig carcasses, reducing the labour and cost of the reference method. The benefits of this technology in pig carcass classification across EU member countries is still being debated (Daumas et al., 2014).

1.7 Automated *in-vivo* image segmentation and image analysis

Other than the traditional uses of CT for diagnostic veterinary medicine, currently, the use of CT in livestock species is primarily concerned with the determination of meat production characteristics in the carcass of live animals and as such, the separation and quantification of different tissues within the carcass are of particular interest.

As part of the CT image analysis routines performed, segmentation of carcass from non-carcass tissues is required to adequately estimate the tissues of interest. This normally requires manually identifying tissue boundaries and removing the internal organs from the image (Figure 1.4), allowing the measurement of tissue densities and areas within the segmented image. However this manual process is time consuming and challenging for the operator and accuracy can also vary between operators. To address these restrictions when

manually segmenting images from sheep, automatic procedures were developed at Scotland's Rural College (SRUC) and Bioinformatics and Statistics Scotland (BioSS), applying mathematical algorithms to identify tissue boundaries. These sheep tomogram analysis routines (STAR) enabled the automatic segmentation and measurement of CT variables routinely and quickly (Glasbey and Young, 2002; Mann et al., 2013; Navajas et al., 2006b).

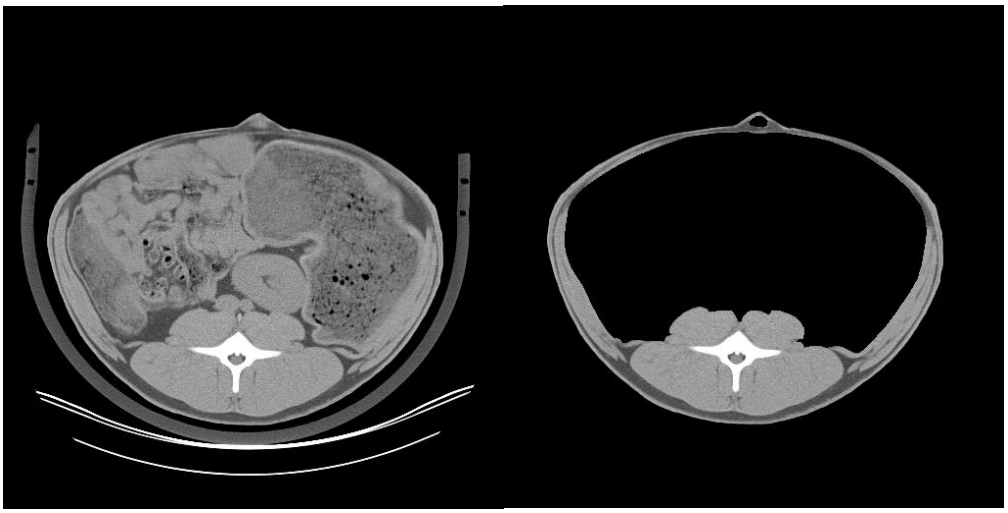


Figure 1.4 Cross-sectional CT image through a lamb *in-vivo* at the 5th lumbar vertebra (left) and the same image following segmentation of the internal organs and CT table and cradle

Further research into the optimisation of scanning procedures for sheep at SRUC, saw the development of 'reference scanning'. The aim of this approach was to reduce scan time, cost and welfare implications, while maximising the accuracy of estimated weights of tissues of interest (carcass lean, fat and bone). The development of this technique employed the use of manual dissection to calibrate the measurement of tissue weights (section 1.5.1), providing breed specific prediction equations for fat, muscle and bone with the highest accuracies ranging from R^2 0.99. 0.98 and 0.89 respectively (Bunger et al., 2011; Macfarlane et al., 2006b; Young et al., 2001).

The reference scanning approach involves collecting CT information for each tissue, such as area, average density, and standard deviation of density, from 3 or 4 cross-sectional tomograms, depending on sheep breed. These reference scan sites are identified from the traditional x-ray topogram (Figure 1.5), in Texel's these are positioned at the ischium bone (i), the fifth lumbar vertebra (ii) and the eighth thoracic vertebra (iii).

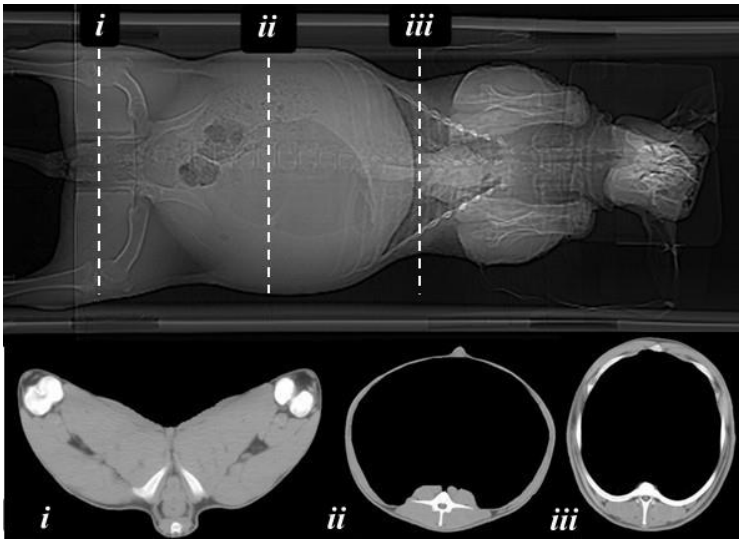


Figure 1.5 Topogram and 2-dimensional cross-sectional CT scans taken in Texel sheep at the ischium (i), 5th lumbar vertebra (ii) and 8th thoracic vertebra (iii)

1.8 The use of Computed tomography in sheep breeding programmes

Computed tomography has been used in terminal sire sheep breeding programmes over the last few decades and in the UK, breeds such as Texel, Suffolk and Charollais (which account for the main terminal sire sheep breeds in the UK as mentioned in section 1.2), as well as breeds such as Hampshire Down, and composite breeds such as the MeatLinc are now routinely scanned.

Very accurate predictions of carcass fat and lean weight are possible using CT, with accuracies of 98% and 99% for lean and fat respectively (Bunger et al., 2011; Macfarlane et al., 2006b; 2001).

Although CT scanning is becoming increasingly accessible, with the use of mobile scanners, and valuable in the routine capture of multiple traits of interest (e.g. lean meat yield, carcass fatness, muscularity) and with high accuracy, the costs of routine scanning are restrictive. The relative high cost associated with CT scanning limits the accessibility of such technology and also the application to large numbers of animals. Instead it is advised, as a cost-effective application of resources, that such technology is applied only as part of a two-stage selection programme. In this scenario, a subset of animals from the wider population (recommended ~15-20%) are selected for CT scanning following initial assessment based on cheaper and more practical methods to estimate carcass composition such as ultrasound (US) scanning (Jopson et al., 1997; Jopson et al., 2004; Lewis and Simm, 2002).

Performance records such as US measurements of both fat and muscle depths are routinely collected on farm and currently used, alongside growth data, as the first-stage selection criteria for terminal sire breeds, some of which then follow this selection with CT scanning for more accurate assessment of carcass composition, estimating fat and muscle weights and muscularity in the gigot.

With these traits currently incorporated into routine scanning procedures within terminal sire breeding programmes, and as a result of this increased accuracy *in vivo*, substantially higher genetic response in weights of fat and muscle in the carcass can be achieved using CT technology (Jopson et al., 2004; Lewis and Simm, 2002; Moore et al., 2011).

The most suitable way to use this technology in breeding programmes to include carcass quality alongside MQ traits has not been fully investigated. This requires genetic parameters, including estimates of heritability for any new CT-predicted traits of MQ and genetic correlations with other relevant traits, which are currently, included in selection indices e.g. ultrasound measures of fat and muscle, CT estimated overall carcass fat and lean,

muscularity, growth etc. These parameters can then be used to optimally design such breeding programmes.

1.9 Aim and Hypotheses

The main aims of the project are to investigate the best image analysis methods, using both two-dimensional and three-dimensional CT information, employing the resulting CT variables to estimate different meat quality traits (intramuscular fat and mechanical shear force). These approaches will then be tested across different genotypes, investigating the potential for the use of CT estimations developed in one breed, applied to divergent breed types.

Further, the project aims to estimate genetic parameters for the best CT predictors of MQ traits in order to develop the basis for the estimation of economically important EBV's for the industry. This may allow EBVs for MQ traits to be included in selection indices alongside other economic traits, such as live weight, carcass fat and muscle weight, muscularity etc. to enable optimal selection strategies to simultaneously improve carcass and meat quality.

The project will use historic data sets to test the following hypotheses and although the data sets did not include meat eating quality data at sufficient levels to robustly test the hypotheses directly with MEQ traits, as outlined in the general introduction, the relationship between MQ traits such as IMF and shear force is sufficient to employ these as proxy traits for MEQ traits.

The project aims to test the hypotheses that:

1. Two and three-Dimensional x-ray computed tomography can provide an accurate method for estimating MQ traits in Texel sheep.

2. Resulting MQ predictors can be incorporated into current sheep breeding programmes, allowing continued improvements in growth and carcass traits, whilst maintaining of improving aspects of MQ

Chapter 2: In vivo prediction of intramuscular fat content and shear force in Texel lamb loins using x-ray computed tomography

2.1 Summary

For the consumer, tenderness, juiciness and flavour are often described as most important for meat eating quality, all of which have a close association with intramuscular fat (IMF). X-ray computed tomography (CT) can measure fat, muscle and bone volumes and weights, in vivo in sheep and CT predictions of carcass composition have been used in UK sheep breeding programmes over the last few decades. This study aimed to determine the most accurate combination of CT variables, either including or excluding CT-predicted total carcass fat weight, to predict IMF percentage and mechanically measured shear force of M. Longissimus lumborum in live Texel lambs.

Including or excluding CT-predicted total carcass fat weight in the model, the prediction of shear force was poor with accuracies ranging from Adj R^2 0.03 – 0.14, using combinations of routinely captured CT variables from multiple or single scans.

As expected, predicted carcass fat weight alone accounted for a moderate amount of the variation (Adj R^2 = 0.51) in IMF. Prediction accuracies were significantly improved ($P<0.05$, Adj $R^2>0.65$) using information on fat and muscle densities measured from three CT reference scans, showing that CT can provide an accurate prediction of IMF in the loin of purebred Texel sheep.

Independent of CT-predicted carcass fat weight, average muscle density measured in a single or multiple scans accounted for a moderate amount of the variation in IMF (Adj R^2 = 0.51-0.60), and accuracies of Adj R^2 = 0.67 were achieved in models including other CT variables, but independent of CT-measured fat areas or predicted fat weights.

2.2 Introduction

It is widely accepted that the fat content of meat plays a significant role in the acceptability of major MQ attributes concerning the consumer as mentioned in chapter 1. Given the

genetic relationship between IMF and carcass fat, and the possible impact on MEQ traits such as tenderness, juiciness and flavour, it has been recognised that there is a need to investigate the possibility of the selection against this positive correlation, allowing breeders to continue to select for lean meat yield and reduced carcass fatness without compromising aspects of MQ and associated MEQ traits related to IMF levels.

To continue to select for improved carcass quality, whilst avoiding any detrimental effects on meat (eating) quality, robust and accurate predictions (preferentially *in vivo*) of IMF and objective *in vivo* measures of MQ such as shear force are needed to inform breeding decisions. CT scanning not only provides information on carcass tissue areas, volumes and weights, but resulting CT muscle density parameters have also been shown to be good predictors of IMF in previous studies (Karamichou et al., 2006; Lambe et al., 2010a; Macfarlane et al., 2005) and could potentially predict other aspects of meat quality.

However, the use of CT variables related to carcass tissue densities for the prediction of meat quality has not been fully investigated. This chapter aims to investigate the use of a range of CT variables related to fat and muscle density values (HU) to optimise the prediction of IMF and shear force in the loins of Texel lambs *in vivo*. Firstly, the main aim was to maximise the prediction accuracy of the MQ traits of interest (IMF and shear force) using all available parameters including various CT methods of carcass fat measurement (subcutaneous and intermuscular) and secondly, given the strong relationship between these carcass fat measures and IMF, investigate prediction accuracies independent of CT carcass fat measurements enabling the consideration of various methods both including CT carcass fat measurements and independent of such CT carcass fat measurements.

2.3 Materials and methods

2.3.1 Experimental animals

All procedures involving animals were approved by an animal ethics committee at Scotland's Rural College (SRUC) and were performed under United Kingdom Home Office licence following the regulations of the Animals (Scientific Procedures) Act 1986.

Data from Texel lambs were available from two previously published studies, these included CT measurements on live lambs pre-slaughter, as well as *post-mortem* laboratory measurements of IMF and shear force data. The first experiment (Exp 1) was conducted over two years (2003-2004) and examined the use of various *in vivo* measurements to predict carcass and meat quality in Texel (n=240) and Scottish Blackface lambs (n=233) (Lambe et al., 2008c). The second experiment (Exp 2) was conducted in 2009 and examined the genotypic effects of the Texel muscling quantitative trait locus (TM-QTL) on carcass and meat quality traits in Texel lambs (n=209), which included data from two research farms, in Scotland and Wales (Lambe et al., 2011). In the present study, only the data from the research farm in Scotland were used (n=370), to reduce possible CT-scanner effects resulting from differences in density value distributions across different scanners. Both Texel data sets were combined to produce one larger data set (Exp 1&2) consisting of the results from the two separate trials over three separate years.

In brief, Exp 1&2 comprises data from pure-bred Texel lambs (n=377) of both sexes (females, entire males) produced over three separate years (2003, 2004 and 2009). Lambs were reared to weaning as either singles (n=184), twins (n=168) or artificially hand reared (n=25). Mean age at CT was 132 days (SD = 21.1, range = 91-202 days), mean live weight was 35.32kg (SD = 4.91, range = 20-49kg).

All lambs were lightly sedated (Rompun®, Bayer animal health, Bayer plc., Newbury, UK) at a dose of 0.1-0.2mg xylazine hydrochloride/kg body weight, and were then secured on their

backs in a cradle before being CT-scanned pre-slaughter using a Siemens Somatom Esprit scanner at the SRUC and Bioinformatics and statistics Scotland (BioSS) CT unit in Edinburgh.

2.3.2 X-ray computed tomography measurements

Two-dimensional (2D) cross-sectional scans (Field of view = 450mm, Resolution = 512x512 pixels) were taken at 3 defined anatomical positions, through the top of the leg at the ischium bone (ISC), the loin at the fifth lumbar vertebra (LV5), and through the chest at the 8th thoracic vertebra (TV8) (see Figure 1.5, chapter 1).

This method of scanning has been defined as 'reference' scanning, optimising the number of images required while maximising accuracy of prediction for carcass tissue weights, with these specific anatomical sites derived from previous calibration trials involving CT, validated against manual dissection (Bunger et al., 2011). Image analyses were performed to separate carcass from non-carcass tissues (Glasbey and Young, 2002) and the density of each pixel (0.77mm^2) in the carcass portion was allocated to fat, muscle or bone, according to density thresholds using sheep tomogram analysis routines (STAR) software developed by SRUC and BioSS (Mann et al., 2013). The thresholds defined were; Fat = -174 to -12HU, Muscle = -10 to 92HU and Bone = 94HU and above. Areas (mm^2) and average densities (HU) of each tissue in each 2D image were calculated, as well as standard deviations for the density values of all pixels allocated to each tissue. A novel soft tissue density, and its standard deviation, were also calculated, combining the information from both fat and muscle tissue densities.

Initial analyses included CT data from all three reference scans. Further analyses were then performed identifying a region of interest (ROI) relating to the anatomical position from where the chemically-extracted IMF and mechanical shear force was measured (*M. Longissimus lumborum*) from a subsample of animals in the dataset (n=100). This involved

three levels of image analysis: (*i*) identifying the LV5 scan as the ROI; (*ii*) performing 'virtual dissection' of the LV5 image to isolate the ROI to the muscles surrounding the spine, including *M. longissimus*, *M. psoas major* and *M. psoas minor*; (*iii*) performing virtual dissection of the LV5 image with the ROI restricted to the right side *M. longissimus* muscle (left side of the image, Figure 2.1).

Carcass fat, as a measure of subcutaneous and intermuscular fat, was also predicted using a breed-specific prediction equation (Texel) developed from previous research (Macfarlane et al., 2006b):

$$Pr\_Cfat(kg) = (-2263 + (LW \times 80.26) + (ISCFA \times 0.21) + (LV5FA \times 0.19) + (TV8FA \times 0.221))/1000$$

Where Pr_Cfat is the CT predicted weight of subcutaneous and intermuscular fat (kg), LW is live weight at CT scanning, ISCFA is the area of pixels allocated as fat in the scan image taken at the ischium region (mm^2), LV5FA is the area of pixels allocated as fat in the scan image taken at the 5^{th} lumbar vertebra region (mm^2) and TV8FA is the area of pixels allocated as fat in the scan image taken at the 8^{th} thoracic vertebra.

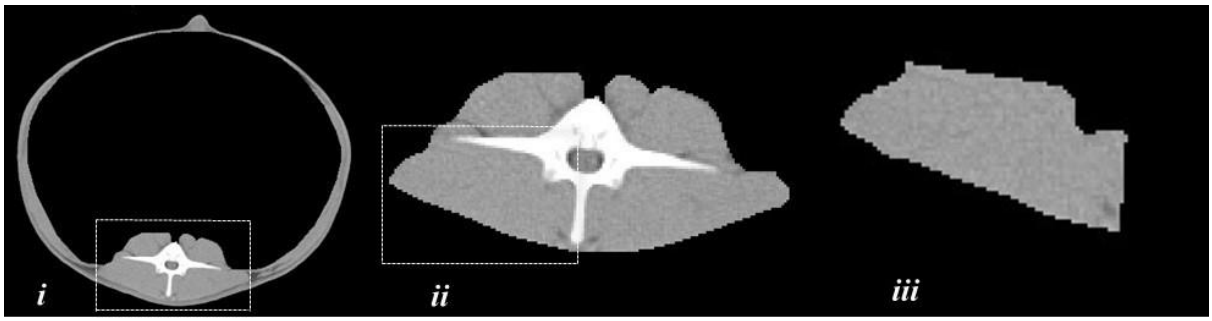


Figure 2.1 Virtual dissections of LV5 scan, LV5 only *(i),* Dissect1 *(ii)* and Dissect2 *(iii)*

2.3.3 Slaughter and meat quality measurements

Mean age at slaughter was 149 days (SD =23.3, range =96-234 days), mean live weight at slaughter was 34.8kg (SD = 5.2, range =19.7-52.2kg). The majority of lambs finished were

slaughtered 4-8 days after CT scanning (n=217), the remaining lambs were slaughtered 32-33 days after CT scanning (n=160), to allow for taste panel analysis after a 30 day withdrawal period from the CT sedative, which formed part of the wider study. Carcasses were chilled for 7 to 9 days and dissected removing the loin muscles (*M. longissimus lumborum*) from the right side of the carcass, which were vacuum-packed, aged for 7 days and frozen. Carcasses included in Exp 2 were subjected to high voltage electrical stimulation at 700 volts RMS for 45 seconds applied between the end of the processing line and the chiller. In both Exp 1 and 2 the muscle samples were transported to the University of Bristol for MQ analyses, chemical IMF was measured in a cross-sectional sample taken from the cranial end of the *M. longissimus lumborum* (at the first lumbar vertebra). Each sample was blended to a fine paste and chemical IMF percentage was measured using petroleum ether (B.P. 40-60^{o}C) as the solvent in a modified Soxhlet extraction (AOAC, 1990).

Shear force was measured using a TA-XT2 texture analyser (Stable Micro System, Surrey, UK) fitted with a Volodkevich-type jaw, a standard compression method to determine tenderness simulating the action of the incisor tooth (Volodkevich, 1938). Loins were cooked 'sous-vide' (in-vacuum-packs) in a water bath at 80^{o}C to an internal core temperature of 78^{o}C (Teye et al., 2006), monitoring individual loin temperature using a digital temperature probe (Hanna Instruments, UK). Samples were then immediately cooled in iced water and held at 4^{o}C overnight for a minimum period of 12 hours. Ten 10 x 10 x 20mm samples were taken across the entire loin following the direction of the muscle fibres and sheared at a constant speed of 1mm/s perpendicular to the muscle fibre direction. Shear force was recorded as the force required (kgF) to shear the sample, with greater values for less tender samples. Results were averaged over the ten samples taken from each loin.

The distribution of both IMF and shear force is shown in Figure 2.2 and a summary of the CT and measured MQ traits can be found in Table 2.1

2.3.4 Statistical analyses

Prior to any statistical analysis, animals without full CT information were removed (n=2), animals with no IMF data were removed (n=2), and finally obvious outliers were identified and animals with chemically-extracted IMF percentages greater than 3 standard deviations from the mean were also removed from the data set (n=3). Initial regression analysis and subsequent model checking (distribution of residuals) suggested the need for transformation of the shear force data. As a result shear force was log-transformed prior to any regression analysis.

Number of days from CT to slaughter (group 1: 4 to 8 days; group 2: 32 to 33 days, to account for those CT scanned early to allow for subsequent taste panel analyses) showed no significant effect on IMF levels or shear force and was therefore not included in the final statistical analyses.

Statistical analyses used simple, multiple and generalized stepwise linear regression in Genstat 14^{TM} (Payne et al., 2012). An adjusted R^2 value (Adj R^2) was used to assess the accuracy of each model, where Adj R^2 = 1-(*residual MS / total MS*), the *total MS* is the sample variance and the *residual MS* is an estimate of σ^2, the variance of a value of Y given the set of X's. The resulting statistic is less biased than unadjusted R^2 (here simply referred to as R^2), and a better measure to use when comparing models with different numbers of predictors. Two approaches were taken in the assessment of prediction models; firstly models inclusive of CT estimated total carcass fat weight (Pr_Cfat) were investigated, and subsequently models excluding Pr_Cfat were investigated.

Furthermore, models were calibrated using a subset of the entire dataset and validated. Available data were split using a natural time series separation in the data (Snee, 1977). Experiment 1 (2003-2004, n=236) data were used as the calibration data set, and Exp 2 (2009, n=134) data were used as the validation data set.

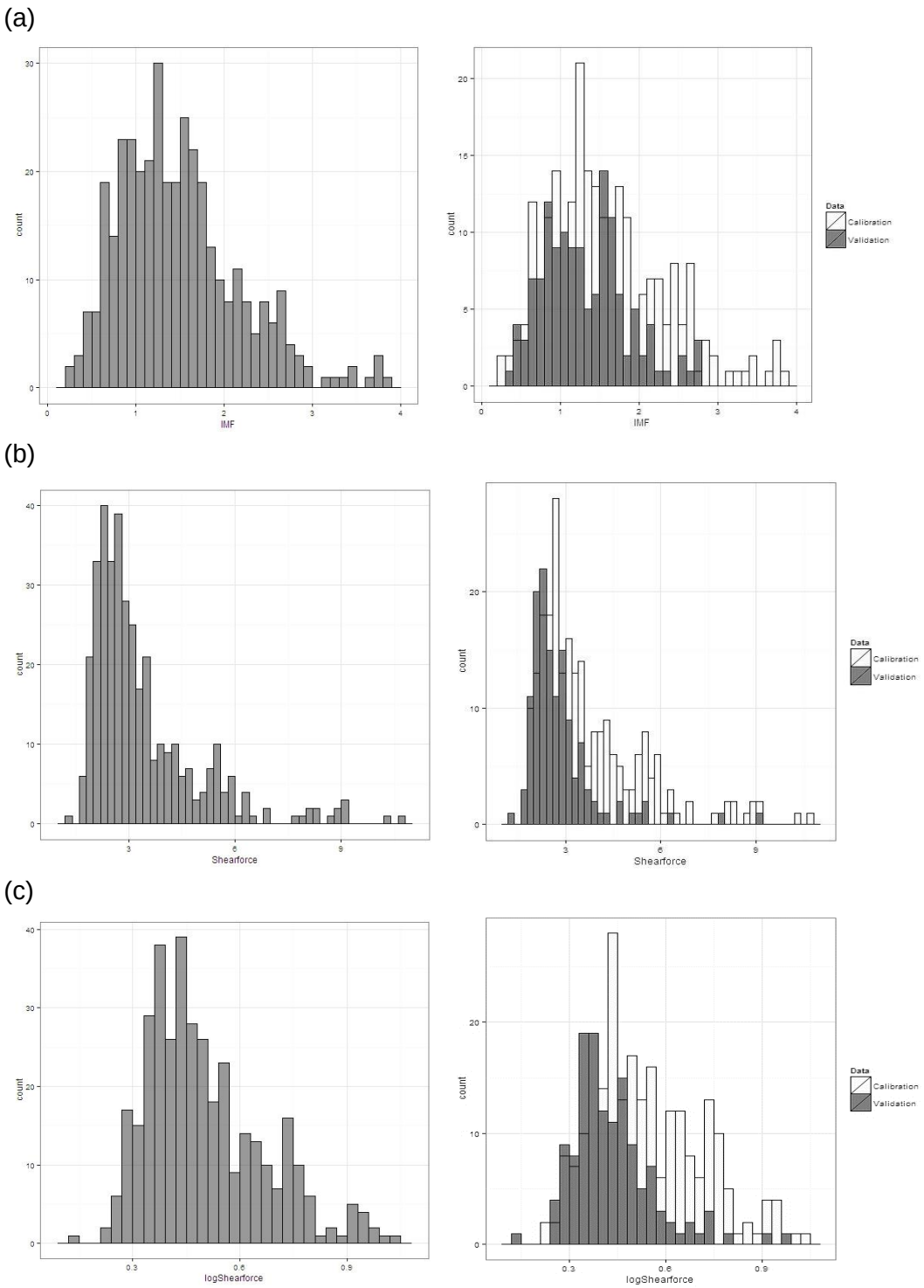


Figure 2.2 Distribution of (a) IMF, (b) shear force, and (c) log transformed (log10) shear force in the loin, from the full data set (n=370; left hand graphs), calibration (n=236) and validation (n=134) data sets (right hand graphs)

Table 2.1 Trait descriptions, means and standard deviations (SD) (n=370)

Trait	Acronym	Trait Description	Mean	SD
CT Traits				
	ISCMD	Average muscle density at ischium scan site (HU)	48.44	2.10
	ISCMSD	Standard deviation of muscle density at ischium scan site (HU)	16.81	0.81
	ISCFD	Average fat density at ischium scan site (HU)	-62.37	5.32
	ISCFSD	Standard deviation of fat density at ischium scan site (HU)	36.51	2.50
	ISCFA	Carcass fat area measured at ischium scan site (mm^2)	3651	1404
	ISCMA	Muscle area measured at ischium scan site (mm^2)	27415	2898
	LV5MD	Average muscle density at 5^{th} lumbar vertebra scan site (HU)	48.30	2.65
	LV5MSD	Standard deviation of muscle density at 5^{th} lumbar vertebra scan site (HU)	18.47	1.67
	LV5FD	Average fat density at 5^{th} lumbar vertebra scan site (HU)	-66.41	7.20
	LV5FSD	Standard deviation of fat density at 5^{th} lumbar vertebra scan site (HU)	42.68	4.35
	LV5FA	Carcass fat area measured at 5^{th} lumbar vertebra scan site (mm^2)	1242	875
	LV5MA	Muscle area measured at 5^{th} lumbar vertebra scan site (mm^2)	9684	1472
	TV8MD	Average muscle density at 8^{th} thoracic vertebra scan site (HU)	44.68	2.98
	TV8MSD	Standard deviation of muscle density at 8^{th} thoracic vertebra scan site (HU)	21.94	1.73
	TV8FD	Average fat density at 8^{th} thoracic vertebra scan site (HU)	-64.64	5.99
	TV8FSD	Standard deviation of fat density at 8^{th} thoracic vertebra scan site (HU)	39.21	3.16
	TV8FA	Carcass fat area measured at 8^{th} thoracic vertebra scan site (mm^2)	3451	1843
	TV8MA	Muscle area measured at 8^{th} thoracic vertebra scan site (mm^2)	12380	1833
	LV5STD	Average soft tissue density at 5^{th} lumbar vertebra scan site (HU)	36.22	8.09
	LV5STSD	Standard deviation of soft tissue density at 5^{th} lumbar vertebra scan site (HU)	40.33	6.19
	ISCSTD	Average soft tissue density at ischium scan site (HU)	35.55	5.07
	ISCSTSD	Standard deviation of soft tissue density at ischium scan site (HU)	40.34	5.66
	TV8STD	Average soft tissue density at 8^{th} thoracic vertebra scan site (HU)	21.84	11.35
	TV8STSD	Standard deviation of soft tissue density at 8^{th} thoracic vertebra scan site (HU)	50.56	6.70
	Pr_Cfat	Predicted total carcass fat weight (kg)	2.34	1.11
MQ Traits				
	Shear force	Mechanically measured shear force in *M. longissimus lumborum* (kgF)	3.40	1.56
	IMF	*M. longissimus lumborum* intra-muscular fat (%)	1.48	0.68

2.3.4.1 Models inclusive of CT estimated carcass fat

CT variables tested in the models to explain variation in IMF and shear force included Pr_Cfat, as well as measurements from the segmented carcass portions of the three CT reference scan sites (ISC, LV5 and TV8; Figure 2.1): muscle area (MA); fat area (FA); average muscle density (MD); average fat density (FD); standard deviation of muscle density (MSD); standard deviation of fat density (FSD); average soft tissue density (STD); and the standard deviation of soft tissue density (STSD).

Phenotypic correlations amongst CT variables and chemically extracted IMF and shear force in the loin were calculated to identify linear relationships between variables (Table 2.2). Given the strong phenotypic relationship between Pr_Cfat and IMF, Pr_Cfat was fitted as a prefix linear variable (indicative for a 'base line' predictor) in all IMF and shear force models.

Subsequent models added CT measurement traits in a progressive manner. Firstly, CT variables from all three cross-sectional scan images, including the novel 'soft tissue' calculation (combining the density ranges between fat and muscle), were used to produce prediction equations for IMF and shear force. Following this, information from the LV5 scan only was used.

To further investigate whether prediction accuracies of IMF could be improved by focusing on the areas of the CT images from which chemical IMF and shear force was actually measured, a virtual sampling method (segmenting regions of interest from the CT images; Figure 2.1) was then considered (IMF only). This involved a random selection of a subset of animals from Exp 1 (n=100 from year 2003). Mean IMF was 1.77% (SD = 0.72), ranging from 0.42 to 3.75%. A summary of measured MQ and CT traits for the subset data employed in the virtual sampling analysis can be found in Table 2.3.

A summary of measured MQ and CT variables for the calibration and validation data can be found in Table 2.4.

Sixteen models were tested in the analysis (Table 2.5). Models using reference data with more than two variables were analysed using stepwise linear regression (Genstat 14^{TM}) to optimize the combination of predictor variables from the maximum model. Models with one or two variables included were analysed using simple or multiple linear regressions, respectively.

2.3.4.2 Models independent of CT estimated carcass fat

CT variables were tested in the models to explain IMF only, these included all CT variables included in the analysis described in section 2.4.1, excluding the use of Pr_Cfat. Given the strong phenotypic relationship between muscle density at the 5th lumbar vertebra (LV5MD) and chemically extracted IMF (r = -0.71), and MD in this CT region was closest to the region of interest with regard to both chemically extracted IMF and shear force, as well as based on previous studies which found MD alone to be a strong predictor of IMF (Karamichou et al., 2006; Lambe et al., 2010a; Navajas et al., 2006a), muscle density (MD) was considered indicative for a base line predictor.

Subsequent models added CT variables in a progressive manner. Again, initially, CT variables from all reference images were used to produce prediction equations for IMF. Following on from this, information from the LV5 scan only was used and models applied in the same progressive manner.

Fifteen models were tested in the analysis independent of Pr_Cfat (Table 2.8). Again models using reference data with more than two variables were analysed using stepwise linear regression (Genstat 14^{TM}). Models with one or two variables included were analysed using simple or multiple linear regressions, respectively.

2.3.4.3 Model validation and selection

Models were then tested for significant differences using their correlation coefficient (√Adj R^2) and applying Fisher's Z transformation (Rasch et al., 1978). To make final model selections between those that predicted IMF and shear force similarly across the whole data set, cross validation analyses were performed. Available data were split using a natural time series separation in the data (Snee, 1977). Experiment 1 (2003-2004, n=236) data was used as the calibration data set, and Exp 2 (2009, n=134) data was used as the validation data set. The fitted terms in the best models derived from the regression analyses of the entire data set were used to produce prediction equations using the calibration data set. These equations were then used to predict the IMF and shear force values of animals included in the validation data. The coefficient of determination (R^2) and error of prediction (RMSEP) were calculated for the predicted IMF percentage and shear force (kgF) in the loin against measured values of both chemically extracted IMF and shear force, to identify the simplest and most reliable single model or group of models.

Table 2.2: Phenotypic correlations amongst CT predictor variables in the reference scans and measured meat quality traits (IMF and shear force). Only correlations significantly greater than zero (P<0.05) are shown

	IMF_Loin	Shear force	Pr_Cfat	ISCMD	ISCMSD	ISCFD	ISCFSD	ISCFA	ISCMA	ISCSTD
IMF_Loin	-									
Shear force	-0.22	-								
PR_Cfat	0.71	-0.18	-							
ISCMD	-0.28	0.14		-						
ISCMSD	0.54	-0.19	0.68		-					
ISCFD	-0.38	0.11	-0.50	-0.18	-0.11	-				
ISCFSD	-0.48		-0.64		-0.50		-			
ISCFA	0.72	-0.16	0.93		0.68	-0.60	-0.63	-		
ISCMA	0.20		0.63	0.33	0.46		-0.47	0.42	-	
ISCSTD	-0.74	0.18	-0.72	0.47	-0.56	0.55	0.48	-0.86		-
ISCSTSD	0.68	-0.14	0.81		0.58	-0.75	-0.48	0.93	0.22	-0.87
	IMF_Loin	Shear force	Pr_Cfat	LV5MD	LV5MSD	LV5FD	LV5FSD	LV5FA	LV5MA	LV5STD
LV5MD	-0.71	0.21	-0.59	-						
LV5MSD	0.66	-0.27	0.68	-0.71	-					
LV5FD	0.47	-0.16	0.45	-0.58	0.71	-				
LV5FSD	-0.71	0.26	-0.83	0.64	-0.79	-0.66	-			
LV5FA	0.71	-0.21	0.90	-0.61	0.69	0.31	-0.85	-		
LV5MA	0.26		0.64	-0.20	0.23	0.35	-0.39	0.41	-	
LV5STD	-0.76	0.22	-0.80	0.77	-0.76	-0.39	0.82	-0.92	-0.23	-
LV5STSD	0.65	-0.22	0.75	-0.57	0.73	-0.27	-0.79	0.90	0.16	-0.94
	IMF_Loin	Shear force	Pr_Cfat	TV8MD	TV8MSD	TV8FD	TV8FSD	TV8FA	TV8MA	TV8STD
TV8MD	-0.72	0.25	-0.58	-						
TV8MSD	0.24	-0.18	0.17	-0.25	-					
TV8FD	-0.37	0.15	-0.51	0.23		-				
TV8FSD	-0.60	0.13	-0.70	0.61	-0.17		-			
TV8FA	0.75	-0.25	0.92	-0.70	0.30	-0.63	-0.66	-		
TV8MA	0.25		0.60	-0.17	-0.25		-0.48	0.42	-	
TV8STD	-0.76	0.26	-0.80	0.80	-0.36	0.60	0.63	-0.93	-0.20	-
TV8STSD	0.65	-0.27	0.73	-0.61	0.47	-0.68	0.53	0.88	0.12	-0.94

Table 2.3: CT and meat quality traits, means and standard deviations (SD) for lambs included in the virtual dissection data set (n=100)

	LV5		Dissect[1]		Dissect[2]	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
MQ Trait						
IMF	1.77	0.72	1.77	0.72	1.77	0.72
Shear force	3.31	1.52	3.31	1.52	3.31	1.52
CT Trait						
LV5FD	-63.86	5.81	-37.66	6.84	-28.12	12.18
LV5FSD	41.23	4.36	22.81	5.16	12.04	8.26
LV5MD	47.52	2.26	55.15	1.68	57.2	1.83
LV5MSD	18.83	1.38	13.72	0.81	11.93	0.85
LV5STD	34.13	8.61	54.19	1.92	56.76	2.04
LV5STSD	41.34	6.84	16.72	1.76	13.42	2.08

LV5; using information from LV5 only, Dissect[1]; using information from dissect[1] CT variables, Dissect[2]; using information from dissect[2] CT variables (see Fig 2.2)

Table 2.4: CT and MQ traits, means and standard deviations for lambs included in the calibration data set (n=236) and validation data set (n=134)

		Calibration Data (n=236)		Validation Data (n=134)	
Trait	**Acronym**	**Mean**	**SD**	**Mean**	**SD**
CT Traits					
	ISCMD	49.32	1.78	46.90	1.69
	ISCMSD	16.87	0.76	16.71	0.89
	ISCFD	-63.48	5.61	-60.43	4.14
	ISCFSD	35.91	1.96	37.57	2.97
	ISCFA	3987	1421	3060	1164
	ISCMA	28318	2492	25823	2887
	LV5MD	48.41	2.62	48.12	2.70
	LV5MSD	18.39	1.67	18.62	1.66
	LV5FD	-65.85	6.61	-67.40	8.07
	LV5FSD	42.26	4.47	43.42	4.03
	LV5FA	1350	982	1051	603
	LV5MA	10160	1319	8846	1350
	TV8MD	44.93	2.97	44.24	2.97
	TV8MSD	21.51	1.68	22.69	1.56
	TV8FD	-64.66	6.57	-64.59	4.84
	TV8FSD	38.50	2.92	40.45	3.18
	TV8FA	3589	1985	3209	1541
	TV8MA	12861	1652	11533	1834
	LV5STD	35.95	8.91	36.71	6.41
	LV5STSD	40.42	6.86	40.17	4.79
	ISCSTD	35.43	5.45	35.77	4.33
	ISCSTSD	41.74	5.89	37.88	4.23
	TV8STD	21.97	12.22	21.62	9.66
	TV8STSD	50.28	7.42	51.06	5.16
	Pr_Cfat	2.60	1.08	1.74	1.01
MQ Traits					
	Shear force				
	IMF	1.58	0.74	1.31	0.54

2.4 Results

2.4.1 Models inclusive of CT estimated carcass fat

2.4.2 Estimating IMF and shear force using reference scan information

Mean IMF was 1.48% (SD = 0.68) and ranged from 0.27 to 3.88%. Mean shear force was 3.4kgF (SD = 1.56, ranging from 1.39-10.72kgF). Pr_Cfat alone accounted for no variation in shear force (Adj R^2 = 0.03). Following stepwise linear regression analysis the accuracy was significantly increased ($P<0.05$, Adj R^2 = 0.10) by also including fat area measured in the ischium (ISCFA) and 8th thoracic vertebra (TV8FA) scans. The accuracy was further improved, but not significantly, to a maximum Adj R^2 = 0.14, with the inclusion of standard deviation of fat density in the ischium and 5th lumbar vertebra scans (ISCFSD, LV5FSD), standard deviation of muscle density in the ischium and 8th thoracic vertebra scans (ISCMSD, TV8MSD), muscle density in the ischium scan (ISCMD) and fat area in the ischium, fifth lumbar vertebra and 8th thoracic vertebra scans (ISCFA, LV5FA, TV8FA). As expected, Pr_Cfat alone accounted for a moderate amount of the variation in IMF (Adj R^2 = 0.51).

For IMF, ten models out of the fifteen models tested (not including the 'baseline') included additional CT variables, with statistically significant improvement in accuracy of prediction when compared to Pr_Cfat as a single predictor ($P<0.05$). Models C^{ref}, D^{ref}, E^{ref}, G^{ref} and I^{ref} were shown not to be significantly different in prediction accuracy from Pr_Cfat (A^{ref}) (Adj R^2 = 0.54, 0.60, 0.57, 0.60 and 0.56 respectively). All other models were >Adj R^2 = 0.63 (Table 2.5).

From these ten models, the model with the highest Adj R^2 value was identified (Model L^{ref}; Adj R^2 = 0.68), which included areas, average densities and density standard deviations for both fat and muscle in the maximum model. The fitted terms included average muscle density from the LV5 and TV8 scans. This model was then used as a benchmark model in

order to compare the ten models identified as better predictors of IMF from reference scan information than Pr_Cfat alone.

Models with statistically significantly lower accuracy ($P<0.05$) compared to the benchmark model (L^{ref}) were discarded. All ten original models identified were retained, however the final fitted terms in models B^{ref} and H^{ref} were identical following the stepwise procedure, and as a result model H^{ref} was discarded. This left nine models (including the benchmark model L^{ref}) with correlation coefficients that were not significantly different from one another, meaning that the prediction ability of these nine models is statistically similar. Therefore, a group of models was identified that would equally well predict IMF using different combinations of reference scan information. These models included M^{ref} (Adj R^2 0.64), B^{ref} (Adj R^2 0.66), F^{ref}, J^{ref}, K^{ref} (Adj R^2 0.67), L^{ref}, N^{ref}, O^{ref}, P^{ref} (Adj R^2 0.68).

2.4.3 Estimating IMF and shear force using LV5 scan information

The use of information from the LV5 scan image only to predict shear force was poor, producing a maximum accuracy of Adj $R^2 = 0.09$ (Table 2.5). Given these low accuracies in the prediction of shear force, further cross validation and progressive image analysis was only carried out in the prediction of IMF. Models using only information from the LV5 scan image to predict IMF were again compared to the simple linear model using only Pr_Cfat and nine models were identified as being significantly more accurate in the prediction of IMF. These models were B^{LV5}, F^{LV5} (Adj R^2 0.63), H^{LV5}, J^{LV5}, N^{LV5}, O^{LV5} (Adj R^2 0.64), K^{LV5}, P^{LV5} (R^2 0.65) and L^{LV5} (Adj R^2 0.66).

Model B^{LV5} and F^{LV5} resulted in the same final fitted terms following the stepwise procedure, so F^{LV5} was discarded, leaving eight final models shown not to be significantly different ($P<0.05$) from the benchmark model (L^{ref}). These eight models were then tested for significance against the model including the largest amount of explanatory variables from the group of models identified as most accurate in explaining the variation of IMF (Model

L^{ref}) in the entire data set. All eight models were retained, as none were shown to be significantly different from model L^{ref} ($P<0.05$).

Table 2.5: Linear regression models between IMF, shear force and CT tissue density parameters including Pr_Cfat, with adjusted coefficient of determination (Adj R2) and residual mean square error (RMSE), based on the whole data set (n=370)

		Shear force (Log10)				IMF			
		Ref[1]		LV5[2]		Ref[1]		LV5[2]	
	Maximum Model	**Adj R^2**	**RMSE**	**Adj R^2**	**RMSE**	**Adj R^2**	**RMSE**	**Adj R^2**	**RMSE**
A	Pr_Cfat	0.03	0.16	0.03	0.16	0.51	0.48	0.51	0.48
B	Pr_Cfat+MD	0.07	0.16	0.05	0.16	0.66[ab]	0.40	0.63[ab]	0.41
C	Pr_Cfat+FD	0.05	0.16	0.04	0.16	0.54	0.47	0.54	0.47
D	Pr_Cfat+MA	0.04	0.16	0.03	0.16	0.60[b]	0.43	0.56	0.45
E	Pr_Cfat+FA	0.10[a]	0.16	0.04	0.16	0.57	0.45	0.53	0.47
F	Pr_Cfat+MD+FD	0.07	0.16	0.05	0.16	0.67[ab]	0.40	0.63[ab]	0.41
G	Pr_Cfat+MA+FA	0.11[a]	0.16	0.05	0.16	0.60[b]	0.43	0.56	0.45
H	Pr_Cfat+MD+MSD	0.09	0.16	0.07	0.16	0.66[ab]	0.40	0.64[ab]	0.41
I	Pr_Cfat+FD+FSD	0.10[a]	0.16	0.07	0.16	0.56	0.46	0.55	0.46
J	Pr_Cfat+MD+MSD+FD+FSD	0.12[a]	0.16	0.09	0.16	0.67[ab]	0.39	0.64[ab]	0.41
K	Pr_Cfat+MD+MSD+FD+FSD+FA	0.14[a]	0.15	0.09	0.16	0.67[ab]	0.39	0.65[ab]	0.41
L	Pr_Cfat+MD+MSD+FD+FSD+MA+FA	0.14[a]	0.15	0.09	0.16	0.68[ab]	0.39	0.66[ab]	0.40
M	Pr_Cfat+STD	0.08	0.16	0.05	0.16	0.64[ab]	0.41	0.60[b]	0.43
N	Pr_Cfat+STD+STSD	0.09	0.16	0.05	0.16	0.68[ab]	0.39	0.64[ab]	0.41
O	Pr_Cfat+STD+STSD+FA	0.10[a]	0.16	0.05	0.16	0.68[ab]	0.39	0.64[ab]	0.41
P	Pr_Cfat+STD+STSD+FA+MA	0.11[a]	0.16	0.05	0.16	0.68[ab]	0.39	0.65[ab]	0.40

Ref[1]; using information from all three reference scans, LV5[2]; using information from LV5 scan only

[a] Adj R^2 values are significantly greater than model A ($P<0.05$)

[b] Adj R^2 values do not differ significantly from model L^{ref} (IMF benchmark)

2.4.4 Estimating IMF using virtually dissected images from a single LV5 scan

Image analysis then considered the use of regions of interest (ROI) taken from the LV5 scan, comparing the use of information from: (i) the full LV5 scan (LV5); (ii) Dissect[1]; or (iii) Dissect[2] (Figure 2.2). Models were again compared using the correlation coefficient of each model and tested for significant differences using Fisher's Z transformation.

There was no significant improvement in accuracy at any stage during the virtual dissection of the LV5 image, and in many cases there was a decrease in accuracy, compared to using data from the full LV5 image, although again not a significant decrease (Table 2.6). Furthermore, there was no significant improvement in the accuracy of the models within ROI method from employing additional information from CT variables.

Table 2.6: Linear regression models between IMF and CT tissue density parameters during virtual dissection, with adjusted coefficient of determination (R2) and residual mean square error (RMSE), based on the subset of the data (n=100)

		LV5		Dissect[1]		Dissect[2]	
	Model	**Adj R^2**	**RMSE**	**Adj R^2**	**RMSE**	**Adj R^2**	**RMSE**
A	Pr_Cfat	0.43	0.54	0.43	0.54	0.43	0.54
B	Pr_Cfat+MD	0.61	0.45	0.54	0.49	0.55	0.48
C	Pr_Cfat+FD	0.47	0.52	0.43	0.54	0.44	0.54
D	Pr_Cfat+MA	0.48	0.52	0.49	0.51	0.49	0.51
E	Pr_Cfat+FA	0.44	0.54	0.43	0.54	0.43	0.54
F	Pr_Cfat+MD+FD	0.61	0.45	0.54	0.49	0.58	0.47
G	Pr_Cfat+MA+FA	0.48	0.52	0.49	0.51	0.49	0.51
H	Pr_Cfat+MD+MSD	0.61	0.45	0.54	0.49	0.55	0.48
I	Pr_Cfat+FD+FSD	0.48	0.52	0.45	0.53	0.46	0.53
J	Pr_Cfat+MD+MSD+FD+FSD	0.61	0.44	0.55	0.48	0.59	0.46
K	Pr_Cfat+MD+MSD+FD+FSD+FA	0.61	0.44	0.55	0.48	0.59	0.46
L	Pr_Cfat+MD+MSD+FD+FSD+MA+FA	0.62	0.44	0.57	0.47	0.62	0.44
M	Pr_Cfat+STD	0.54	0.49	0.52	0.50	0.53	0.49
N	Pr_Cfat+STD+STSD	0.56	0.47	0.54	0.48	0.55	0.48
O	Pr_Cfat+STD+STSD+FA	0.60	0.45	0.55	0.48	0.55	0.48
P	Pr_Cfat+STD+STSD+FA+MA	0.61	0.45	0.58	0.47	0.58	0.47

LV5; Using information from LV5 only, Dissect[1]; using information from dissect1 CT variables, Dissect [2]; using information from dissect2 CT variables

2.4.5 Model validation and selection

These analyses identified seventeen models that were shown to be statistically similar in their prediction accuracies of IMF, including either information from the reference scans or LV5 scan only.

The final seventeen models identified were then used to perform cross validation analysis. Seventeen prediction equations were derived using the validation data set, corresponding to the seventeen 'best' models identified from primary analysis (Table 2.7). The models were then used to predict the IMF values of animals included in the validation data. Coefficients

of determination (R^2) and error of prediction (RMSEP) for the predicted IMF percentage in the loin against chemically extracted IMF are also shown in Table 2.7.

The models with the strongest cross validity were models M^{ref} (R^2_{cal} 0.64, R^2_{val} 0.67) and N^{ref} (R^2_{cal} 0.67, R^2_{val} 0.67), using soft tissue density information from all three reference scans (M^{ref}) and using soft tissue density information from the LV5 and TV8 scans alongside the standard deviation of soft tissue density from all three reference scans (N^{ref}). Residual mean square error of prediction (RMSEP) in the validation data compared to the calibration data, decreased slightly across all models. The reduction of RMSEP is due to the characteristics of the validation data set (Figure 2.3). The reduction in variation of IMF across the validation data set reduces the error of the prediction. These models were then used as a benchmark and all other models were tested for significant differences in correlation coefficients using Fisher's Z transformation (Rasch et al., 1978). All seventeen models were found to be statistically similar in prediction accuracy ($P<0.05$) and no significant reduction in prediction accuracy was seen across the calibration and validation models.

From this, two models were chosen from the criteria of firstly, the simplest and best models (N^{ref}) and the simplest model that was shown to be significantly more accurate in prediction than the baseline (B^{ref}). Final models are shown below.

Pr_IMF_Bref (%) = 6.920 + (Pr_Cfat*0.2425) - (LV5MD*0.0654) – (TV8MD*0.0637)

Pr_IMF_Nref (%) = 7.320 + (Pr_Cfat*0.0565) – (LV5STD*0.0626) – (TV8STD*0.03585) + (ISCSTSD*0.02209) – (LV5STSD*0.0565) – (TV8STSD*0.0303)

Table 2.7: Linear regression models between IMF and CT tissue density parameters including Pr_Cfat, with adjusted coefficient of determination (R2) and residual mean square error (RMSE), based on the training data set (n=236) and validation data set (n=134)

		Calibration (n=236)		Validation (n=134)	
	Fitted Terms	**Adj R^2**	**RMSE**	**Adj R^2**	**RMSEP**
B^{ref}	Pr_Cfat, LV5MD, TV8MD	0.69	0.41	0.63	0.33
F^{ref}	Pr_Cfat, LV5MD, TV8MD, ISCFD	0.69	0.41	0.63	0.33
J^{ref}	Pr_Cfat, LV5MD, TV8MD, ISCFD, LV5FSD	0.69	0.41	0.64	0.32
K^{ref}	Pr_Cfat, LV5MD, TV8MD, ISCFA, LV5FA	0.70	0.41	0.63	0.32
L^{ref}	Pr_Cfat, LV5MD, TV8MD, LV5FD, ISCMA, LV5FA, TV8FA	0.71	0.41	0.65	0.32
M^{ref}	Pr_Cfat, ISCSTD, LV5STD, TV8STD	0.64	0.45	0.67	0.31
N^{ref}	Pr_Cfat, LV5STD, TV8STD, ISCSTSD, LV5STSD, TV8STSD	0.67	0.42	0.67	0.30
O^{ref}	Pr_Cfat, ISCSTD, ISCSTSD, LV5STD, LV5STSD, TV8STD, ISCFA, TV8FA, ISCFA	0.68	0.42	0.66	0.31
P^{ref}	Pr_Cfat, LV5STD, LV5STSD, TV8STD, TV8STSD, ISCMA, TV8FA	0.69	0.41	0.66	0.31
B^{LV5}	Pr_Cfat, LV5MD	0.67	0.43	0.57	0.35
H^{LV5}	Pr_Cfat, LV5MD, LV5MSD	0.67	0.43	0.59	0.35
J^{LV5}	Pr_Cfat, LV5MD, LV5FD, LV5FSD	0.68	0.42	0.57	0.35
K^{LV5}	Pr_Cfat, LV5MD, LV5FSD, LV5FA	0.68	0.42	0.59	0.35
L^{LV5}	Pr_Cfat, LV5MD, LV5FD, LV5MA, LV5FA	0.68	0.42	0.60	0.34
N^{LV5}	Pr_Cfat, LV5STD, LV5STSD	0.64	0.44	0.61	0.34
O^{LV5}	Pr_Cfat, LV5STD, LV5STSD, LV5FA	0.64	0.44	0.61	0.34
P^{LV5}	Pr_Cfat, LV5STD, LV5STSD, LV5MA	0.66	0.43	0.62	0.33

2.4.6 Models independent of CT estimated carcass fat

Given the poor results obtained during the previous analyses to predict shear force from CT information using any of the methods, or to predict IMF using virtual dissection in the LV5 image, it was decided to concentrate only on the prediction of IMF in the loin and investigate using reference information and LV5 only.

2.4.7 Estimating IMF using reference scan information

As expected, MD in the reference scans accounted for a moderate amount of variation in IMF (Adj R^2 = 0.60). There was no model, from the 14 models tested not including the baseline model (MD), with statistically significant improvement in prediction accuracy,

however five models were significantly lower in in accuracy of prediction when compared to the baseline model ($P<0.05$). Models B^{ref_ex}, C^{ref_ex}, D^{ref_ex}, F^{ref_ex} and H^{ref_ex} were therefore dropped from further analysis.

From the remaining ten models, the model with the highest Adj R^2 value was chosen as a benchmark model (Model K^{ref_ex}; Adj $R^2 = 0.68$), all remaining models were tested for significant differences in prediction accuracy to the benchmark (K^{ref_ex}). All ten models were shown to have no significant difference in accuracy ($P<0.05$), however, following the stepwise procedure during the regression analysis, final parameters fitted to model M^{ref_ex}, N^{ref_ex} and O^{ref_ex} were identical and as a result models N^{ref_ex} and O^{ref_ex} were discarded. The remaining eight models included A^{ref_ex} (Adj $R^2 = 0.60$), E^{ref_ex}, L^{ref_ex} (Adj $R^2 = 0.63$), G^{ref_ex} (Adj $R^2 = 0.61$), I^{ref_ex} (Adj $R^2 = 0.66$), K^{ref_ex} (Adj $R^2 = 0.68$) and M^{ref_ex} (Adj $R^2 = 0.67$) all not significantly different in their prediction ability (Table 2.8).

2.4.8 Estimating IMF using LV5 scan information

Models using the CT parameter information from the LV5 scan only were again compared to the baseline model including only LV5MD. A moderate amount of the variation in IMF could be explained by the use of LV5MD alone (Adj $R^2 = 0.51$). Six models were found to be significantly more accurate in the prediction IMF. These models were M^{LV5_ex} (Adj $R^2 = 0.61$), I^{LV5_ex}, N^{LV5_ex}, O^{LV5_ex} (Adj $R^2 = 0.62$), J^{LV5_ex} and K^{LV5_ex} (Adj $R^2 = 0.64$).

Model N^{LV5_ex} and O^{LV5_ex} included the same CT variables in the final fitted models following the stepwise procedure, therefore model O^{LV5_ex} was discarded. Models were then subsequently tested for significant differences against the benchmark model (K^{LV5_ex}), chosen on Adj R^2 value and number of parameters included in the model as previously explained. All models were retained, as none were shown to be significantly different from model K^{LV5_ex} ($P<0.05$) (Table 2.7). Model L^{LV5_ex} was not significantly greater in prediction accuracy than the baseline (A^{LV5_ex}), however it was also not significantly different in

accuracy from the benchmark model (K^{LV5_ex}) (Table 2.7). As a result of this, and because there were a number of models available, model L^{LV5_ex} was discarded.

Table 2.8: Linear regression models between IMF CT tissue density parameters in models excluding Pr_Cfat, with adjusted coefficient of determination (Adj R2) and residual mean square error (RMSE), based on the whole data set (n=370)

		IMF			
		Ref[1]		LV5[2]	
	Maximum Model	**Adj R^2**	**RMSE**	**Adj R^2**	**RMSE**
A	MD	0.60[b]	0.44	0.51	0.48
B	FD	0.40	0.53	0.22	0.60
C	MA	0.07	0.66	0.07	0.66
D	FA	0.57	0.45	0.51	0.48
E	FD	0.63[b]	0.42	0.22	0.60
F	MA+FA	0.58	0.44	0.51	0.48
G	MD+MSD	0.61[b]	0.43	0.55	0.46
H	FD+FSD	0.53	0.47	0.50	0.48
I	MD+MSD+FD+FSD	0.66[b]	0.40	0.62[ab]	0.42
J	MD+MSD+FD+FSD+FA	0.67[b]	0.39	0.64[ab]	0.41
K	MD+MSD+FD+FSD+FA+MA	0.68[b]	0.39	0.64[ab]	0.41
L	STD	0.63[b]	0.42	0.58[b]	0.45
M	STD+STSD	0.67[b]	0.39	0.61[ab]	0.43
N	STD+STSD+FA	0.67[b]	0.39	0.62[ab]	0.42
O	STD+STSD+FA+MA	0.67[b]	0.39	0.62[ab]	0.42

Ref[1]; using information from all three reference scans, LV5[2]; using information from LV5 scan only

[a] Adj R^2 values are significantly greater than model A ($P<0.05$)

[b] Adj R^2 values do not differ significantly from model K^{ref} (IMF benchmark)

2.4.9 Model validation and selection

The analysis of models using both entire reference scan information and LV5 scan only information identified thirteen potential models in the prediction of IMF. All thirteen models were statistically similar in accuracy. These final thirteen models were then cross validated using the same time series data split, and the same calibration and validation data sets as described above. Thirteen prediction equations were derived using the calibration data set (Table 2.8). The models were again used to predict the IMF values of animal included in the validation data. Coefficients of determination (R^2) and error of prediction (RMSEP) are shown in Table 2.8. The model with the strongest cross validity was model M^{ref_ex} (R^2_{cal} 0.68,

R^2_{val} 0.67), using soft tissue density information from all three reference scans. However, no model's validation accuracy fell significantly when compared to calibration accuracies ($P<0.05$).

It was also recognised that not all models were entirely independent of the amount of CT carcass fat in the lamb, as, although Pr_Cfat was not included in the models, some models in the analysis included CT-measured fat areas (FA) so were not independent of overall amount of carcass fat. Therefore, models J^{ref_ex}, K^{ref_ex}, J^{LV5_ex}, K^{LV5_ex} and N^{LV5_ex} were not considered for selection. From the remaining models, one model was selected on the basis of the single best model employing CT parameter information which is routinely collected during current practices at SRUC (I^{ref_ex}) using information from the reference scans, including MD, MSD, FD and FSD. The final selected model is shown below (Adj R^2 = 0.67 with the full data set):

Pr_IMF_Iref_ex (%) = 7.26-(0.0720*LV5MD) - (0.0611*TV8MD) + (0.0748*ISCMSD) - (0.02090*ISCFD) - (0.00758*LV5FD) - (0.0344*ISCFSD) - (0.0324*LV5FSD)

Table 2.9: Linear regression models between IMF and CT tissue density parameters excluding Pr_Cfat, with adjusted coefficient of determination (R2) and residual mean square error (RMSE), based on the training data set (n=236) and validation data set (n=134)

		Calibration (n=236)		Validation (n=134)	
	Fitted Terms	**Adj R^2**	**RMSE**	**Adj R^2**	**RMSE**
A^{ref_ex}	ISCMD, LV5MD, TV8MD	0.65	0.44	0.56	0.36
E^{ref_ex}	ISCMD, LV5MD, TV8MD, ISCFD, LV5FD, TV8FD	0.67	0.42	0.59	0.34
G^{ref_ex}	ISCMD, LV5MD, TV8MD, ISCMSD, LV5MSD	0.67	0.42	0.63	0.33
I^{ref_ex}	LV5MD, TV8MD, ISCMSD, ISCFD, LV5FD, ISCFSD, LV5FSD	0.70	0.41	0.63	0.33
J^{ref_ex}	LV5MD, TV8MD, LV5FD, ISCFA, LV5FA	0.70	0.41	0.64	0.32
K^{ref_ex}	LV5MD, TV8MD, LV5FD, ISCFA, LV5FA, ISCMA, TV8MA	0.70	0.40	0.65	0.32
L^{ref_ex}	ISCSTD, LV5STD, TV8STD	0.64	0.45	0.66	0.31
M^{ref_ex}	LV5STD, TV8STD, ISCSTSD, LV5STSD, TV8STSD	0.68	0.42	0.67	0.31
I^{LV5_ex}	LV5MD, LV5MSD, LV5FD, LV5FSD	0.67	0.42	0.57	0.35
J^{LV5_ex}	LV5MD, LV5FD, LV5FA	0.67	0.42	0.57	0.35
K^{LV5_ex}	LV5MD, LV5FD, LV5FA, LV5MA	0.68	0.42	0.58	0.35
M^{LV5_ex}	LV5STD, LV5STSD	0.63	0.45	0.59	0.34
N^{LV5_ex}	LV5STD, LV5STSD, LV5FA	0.63	0.45	0.59	0.34

2.5 Discussion

The prediction of shear force using CT derived information could not be achieved at a satisfactory level of accuracy. Other post-mortem and conditioning factors, such as muscle fibre type and size, cooking loss, ultimate pH, and post-mortem glycolysis play an important role in the conversion of muscle to meat, and may have significant effects on shear force. The use of parameters measured by computed tomography *in vivo*, to produce prediction equations for shear force in a processed, aged and cooked piece of meat, may be too far removed for the successful estimation of mechanical shear force.

A minimum level of 3% chemically extracted intramuscular fat in grilled cuts of lamb, ensuring consumer acceptability, was recommended following a review of the literature by

Savell and Cross (1988). During this current study the mean IMF level reported for purebred Texel was 1.48%, well below the level recommended by Savell and Cross. At a similar end point mean IMF in Scottish Blackface lambs was reported as 2.3% (Lambe et al., 2008c). In an Australian study involving crossbred lambs from several Terminal, Maternal and Merino sires, mean IMF was 4.1% in females and 4.2% in males, whilst mean IMF values of 4.3%, 4.5% and 4.1% were recorded in lambs from the Maternal, Merino and Terminal sires, respectively (Pannier et al., 2014a), providing evidence of breed differences when considering IMF levels in sheep.

It has been shown in previous studies that muscle density information from single or multiple CT scans taken along the body of sheep *in vivo* can provide moderately accurate predictions of IMF contained within *M. longissimus* of different sheep breeds at finishing. Published prediction accuracies include $R^2 = 0.33$ using muscle density information from reference scan images in Scottish Blackface sheep (Karamichou et al., 2006), $R^2 = 0.36$ employing information from a cross-sectional scan in the 5^{th} lumbar vertebra of purebred Texel sheep (only using muscle density) and in the same study a reasonable improvement in accuracy was shown when fat density and standard deviations of both fat and muscle density were added to the model ($R^2 = 0.48$) (Lambe et al., 2010a). Macfarlane et al. (2005) used similar lean tissue measurements, alongside fat area measurements, in a single cross-sectional scan at the 2^{nd} lumbar vertebra, resulting in a moderate prediction accuracy of $R^2 = 0.57$. These previous studies have shown the possibilities of using CT scanning as a predictor of IMF in different sheep breeds.

This current study did not fully investigate the effect that other fat deposits, such as intrafibre lipid within the muscle cell, may have on CT measured muscle density. The chemical extraction method used to measure IMF was not sufficient to provide such information. The use of different statistical approaches, such as partial least squares (PLS) regression compared to ordinary least squares (OLS) regression, has also been investigated in the

prediction of IMF in pig loins (*post-mortem* and *in vivo*), alongside the use of combinations of different x-ray intensities and slice thicknesses. These methods, applied *post-mortem*, have achieved prediction accuracies ranging from R^2 = 0.63-0.80 (Font-i-Furnols et al., 2013). A similar approach *in vivo* achieved maximum prediction accuracies of R^2 = 0.53, with poor results during validation of the models (maximum R^2 = 0.18) (Kongsro and Gjerlaug-Enger, 2013).

The CT settings and methods chosen for image analysis and statistics (e.g. Partial least squares regression (PLS)) can help to deal with the partial volume effect of mixed pixels (pixels consisting of a mixture of fat and muscle), when considering IMF. For example, different x-ray intensities can change the contrast observed between soft tissues. In terms of statistical treatment of the data, the PLS approach considers the proportion of pixels allocated to each HU value, whereas OLS employs information across a range of HU values within defined thresholds. Font-i-Furnols *et al*. (2013) found that the estimation of IMF in pork loins *post-mortem* is better predicted by OLR than PLS regression, and that a reduced x-ray intensity (increasing contrast) was more accurate, however combining information from high and low intensity improved the prediction accuracy further.
There may be scope for very complex approaches to explain the variation in IMF using CT data. However, this study provides evidence that the use of relatively simple means and standard deviations of the CT variables routinely captured can be used to predict IMF in the loin of Texel sheep with moderately high accuracy and allows the retrospective calculation of such a novel trait within existing datasets.

The results from this study also show that further improvements are possible in the prediction of intramuscular fat *in vivo* compared to results from similar previous studies (Karamichou et al., 2006; Lambe et al., 2010a; Macfarlane et al., 2005), with the use of additional information from multiple cross-sectional scans. These results show that muscle density is a good predictor of intramuscular fat, agreeing with previous literature sources,

and the strong phenotypic relationship between CT predicted carcass fat and intramuscular fat provides some improvement in the accuracy of prediction over that of CT muscle density parameters alone. In this study the addition of standard deviation of muscle density did not significantly improve the accuracy of prediction, as it did in previous studies (Lambe et al., 2010a). The inclusion of the standard deviation of soft tissue density resulted in a slight increase in accuracy. The biological relationship between the standard deviation of soft tissue density and intramuscular fat is not fully understood and requires further analysis. It appears that the distribution of muscle and fat pixels changes and the proportion of 'mixed' pixels (pixels containing both fat and muscle density values according to thresholds) within the soft tissue distributions increases in animals with higher levels of IMF. This equates to an increase in mixed pixels with average density values closer to fat thresholds, indicating that they may contain an increased proportion of fat within the pixel area, reducing the average density value within the pixel, although the overall density remains within the muscle thresholds. There is a possibility that the standard deviation of muscle, fat or soft tissue may also produce some clarity in the models when dealing with the partial volume effect mentioned previously.

The inclusion of CT-predicted carcass fat understandably increases the accuracy of prediction of IMF when included in the model and was specifically chosen as a prefix to models and initial benchmark as a single predictor variable due to the strong phenotypic relationship between CT predicted carcass fat and chemically extracted intramuscular fat ($r = 0.71$). An increase in accuracy of prediction of IMF from additional parameters, above that provided by CT predicted carcass fat, is necessary to enable selection for IMF while maintaining or further reducing overall carcass fat. Including both carcass fat and IMF predictors as selection criteria in a multi-trait selection index would then allow simultaneous selection for each fat trait in opposite directions. However to better understand this relationship between carcass fat and IMF, models independent of carcass fat will be taken

forward into the genetic analysis of current ultrasound, CT traits and novel predictions of IMF.

The virtual dissection, and use of information from a defined ROI of the loin, did not improve the accuracy of prediction. This intuitive approach to analyse closer to the area from which chemical IMF was measured proved unsuccessful and suggests that there is valuable muscle and fat density information within the carcass portion of the scan images from other muscles, muscle groups and fat depots that provides an increase in accuracy. This may be further acknowledged given the prediction ability of CT predicted carcass fat information.

The results from this study show that there may be several possible models that have the potential to be used in the prediction of IMF in the loin, with similar levels of accuracy, indicating a possible ceiling in the prediction accuracy of models employing CT variables included in this study. Choosing between such groups of models proves difficult. However a choice may be made when considering the best model; employing information on soft tissue density and its standard deviation (N_{ref}, Adj $R_2 = 0.68$), and the simplest model that was not significantly different in accuracy; employing muscle density information from two of the three reference scan sites (B_{ref}, Adj $R_2 = 0.66$), with the inclusion of CT-predicted carcass fat in both cases.

Successful accuracies were also achieved in models excluding CT-predicted carcass fat, and again these choices between models were difficult to make. However choices were made considering the single best model which employed CT information which is currently collected as part of routine measurements at the SRUC-BioSS CT unit in Edinburgh, this choice was made to both ensure a definitive choice and also to minimise the additional parameter estimation or image analysis involved.

This enables us to predict IMF in sheep with the best accuracy using a vast amount of historic CT data, obtained at SRUC's CT unit over the last 15 years, providing powerful data

sets to estimate heritabilities for CT-predicted IMF and genetic correlations to other production traits. Estimation of these genetic parameters is a required pre-requisite for the integration of this new trait into breeding programmes. Existing studies involving different sheep breeds (Lorentzen and Vangen, 2012) have reported moderate heritability's of IMF in crossbred progeny of Norwegian White ewes crossed with Texel terminal sires ($h^2 = 0.48$, s.e. = 0.16), suggesting that selection for increased IMF should be successful.

The result of this study implies that these further studies of the genetic parameters consider the genetic correlations between models including Pr_Cfat and models not including Pr_Cfat.

Further investigations into the application of CT measurements in the prediction of IMF across other sheep breeds is also required to determine whether across-breed prediction equations would be applicable or breed-specific prediction equations would require development, given that the breed used in this study (Texel) is particularly lean and other breeds may be higher in average IMF levels with greater variation.

2.6 Conclusion

The *in vivo* prediction of IMF in the loin of purebred Texel sheep is possible using CT-derived information on muscle density from one single scan across the loin region of the animal, alongside CT-predicted total carcass fat. The accuracy of prediction can be further increased employing information from additional two-dimensional anatomical reference scans, and by also considering fat density and standard deviations of tissue density values, although these increases in accuracy are not always significant. Some of the more complex models including information related to the soft tissue parameters (combining muscle and fat) appear to be more robust at predicting IMF across different populations. This method may now be applied to a powerful dataset to estimate genetic parameters, allowing judgment of how to improve IMF genetically without compromising important production traits

including carcass quality. This chapter also only investigated the use of simple ‘step and shoot’ two-dimensional imaging, however in the following chapter the use of more advanced three-dimensional spiral computed tomography techniques, and any potential for further increases in accuracy are investigated.

Chapter 3: Prediction of intramuscular fat content and shear force in Texel lamb loins using combinations of different *in vivo* x-ray computed tomography (CT) scanning techniques

3.1 Summary

Computed tomography (CT) parameters, including spiral computed tomography scanning (SCTS) parameters, were related to intramuscular fat (IMF) and mechanically measured shear force measurements using data from two previously published studies. Purebred Texels (n = 377) of both sexes, females (n = 206) and intact males (n = 171), were CT scanned pre-slaughter, at a mean age of 132 d (SD 21.1, range 91-202 d) and mean live weight of 35 kg (SD 4.9, range 20-49 kg). CT-derived tissue area and density information from two- and three-dimensional (SCTS) images were available. These data were related to mechanical shear force (mean 3.4 kg, SD 1.56, range 1.39-10.72 kg) and chemically extracted IMF (Mean 1.48%, SD 0.68, range 0.27-3.88%) data, to investigate the ability of in vivo CT to predict these meat quality traits. Accuracies in the prediction of shear force ranged from R^2 = 0.02, RMSE 0.16 (P = 0.002) to R^2 = 0.13, RMSE = 0.15 (P < 0.001) and for IMF from R^2 = 0.51, RMSE = 0.48 (P < 0.001) to R^2 = 0.71, RMSE = 0.37 (P < 0.001), using different combinations of SCTS information and two-dimensional CT scan information. The prediction of mechanical shear force could not be achieved at an acceptable level of accuracy employing information from CT, even when including SCTS information. However, the prediction of IMF in the loin was promising when employing information from CT, although models including SCTS parameters did not significantly improve previous prediction accuracies achieved using only two-dimensional CT data. The results provide further evidence that a good prediction of IMF for Texel lambs in vivo can be achieved using CT technology, but that the extra expense and image analyses required for three-dimensional SCTS methods may not be justified in the prediction of this trait. This study indicates that there may be several available models of varying degrees of complexity using SCTS technology to predict IMF as a meat quality trait and a proxy for meat eating quality traits (e.g. tenderness, juiciness and flavour). Therefore the application of CT predicted meat quality traits such as IMF can and should be included into current breeding

programmes to allow for the simultaneous selection of animals with desirable production, carcass and meat (eating) quality traits.

3.2 Introduction

The prediction of aspects of meat quality have been investigated both *in vivo* and *post mortem* in meat producing species (Bunger et al., 2011; Font-i-Furnols et al., 2013; Kongsro and Gjerlaug-Enger, 2013; Macfarlane et al., 2005; Prieto et al., 2010). The first method of image capture most commonly used is 'single-slice' scanning. X-rays are used to generate cross-sectional, two-dimensional images of the selected region of a subject. Each image is produced by rotation of the x-ray tube 360^{o} around the subject. Attenuation of radiation through the tissues can then be measured, with differences indicating different tissue densities. Advances in scanning technology have resulted in the development of contiguous scanning procedures such as SCTS, capable of producing a series of images in a single contiguous scan at intervals of as little as 0.6mm apart.

Muscle density information from single or multiple CT scans in sheep, can provide moderately accurate predictions of IMF content *in vivo*. The prediction of other meat quality traits, such as shear force, *in vivo* using CT technology has not been investigated fully, but available results to date are not promising (Chapter 2). Accuracies for IMF range from R^2 = 0.33 to 0.68 including the work carried out in chapter 2 and also including previous studies involving various CT variables (Karamichou et al., 2006; Lambe et al., 2010a; Lambe et al., 2008c; Macfarlane et al., 2005). Accuracies for shear force range from R^2 = 0.14 to 0.35 using combinations of live animal measurements (Lambe et al., 2008c). The aim of this study was to test the hypothesis that SCTS techniques can provide improvements compared to published accuracies using two-dimensional CT information in the prediction of IMF content and shear force in the loins of Texel sheep.

3.3 Materials and methods

3.3.1 Experimental animals

A full description of the datasets and detailed animal procedures can be found in chapter 2, section 2.3.1. In brief, CT variables, including SCTS parameters were measured on live lambs pre-slaughter, as well as *post-mortem* laboratory measurements of IMF and shear force in the loin. Lambs were CT scanned and slaughtered at finishing (ready for slaughter depending on condition score and live weight).

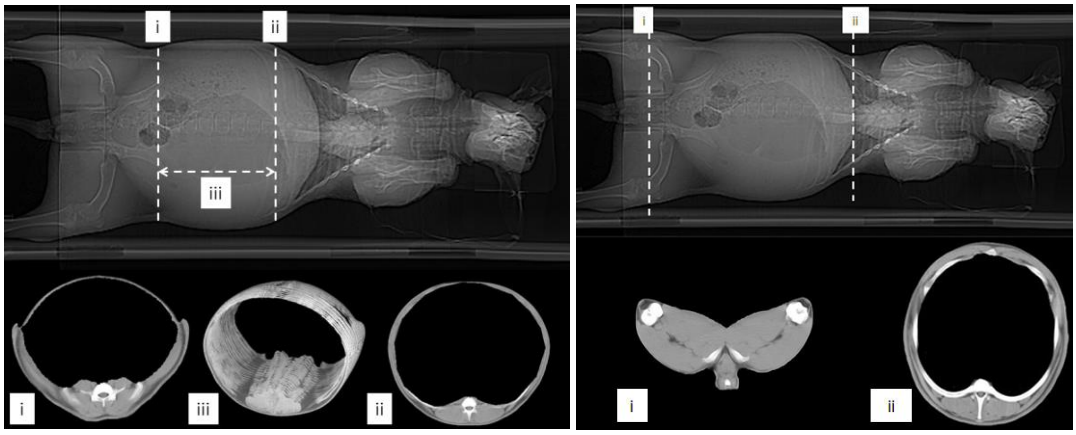
3.3.2 Single-slice and spiral x-ray CT measurements and image analysis

A series of spiral CT images were selected from the loin region of each lamb. The first image was taken where the transverse process of the 7th lumbar vertebra appears and the last image in the series where the transverse process of the 1st lumbar vertebra is no longer visible (Figure 3.1a). Two-dimensional cross-sectional single-slice scans were also used, taken at two defined anatomical positions, through the top of the leg at the ischium bone (ISC), and through the chest at the 8th thoracic vertebra (TV8), details of the images used and the location are presented in Figure 3.1b.

This two dimensional method of scanning at these particular anatomical sites (including an additional scan at the 5th lumbar vertebra, which was not used in this study), is currently used in UK terminal sire breeding programmes to provide accurate predictions of fat and muscle weights in the carcass. This method, defined as 'reference' scanning (Bunger et al., 2011), optimizes the number of images required to be taken across the body of the sheep while maximizing the accuracy of estimations for carcass traits. Images were produced with a resolution of 512 x 512 pixels and a 450mm field of view, producing images with a pixel

size of 0.77mm^2 in two dimensions. Spiral images were produced at the same resolution and field of view at intervals of 8mm, producing images with a voxel size of 6.2mm^3.

Automated analyses were performed on the images produced, to separate carcass from non-carcass tissues (Glasbey and Young, 2002), and to calculate the density of each pixel in Hounsfield units (HU), the standard quantitative scale for describing radio density. In the final segmented images each pixel was allocated to fat, muscle or bone using image thresholding techniques based on these density values (Mann et al., 2003). Areas (mm^2) and average densities (HU) of pixels allocated as muscle and fat in each two dimensional image were calculated, as well as standard deviations of the density values allocated to each tissue. Combining all pixels allocated as either fat or muscle enabled the use of a novel average 'soft tissue density' and its standard deviation. The SCTS images were used to calculate weighted average densities of muscle, fat and soft tissue (average tissue density, in each individual scan image, weighted for tissue area in that image and averaged across all images in the spiral scan series). Volumes of each tissue (mm^3) were also calculated. The resulting SCTS parameters included: weighted average muscle and fat densities and the related standard deviations; weighted average soft tissue density and standard deviation; and calculated muscle and fat volumes (mm^3). The CT variables measured from the two-dimensional single-slice scans in the ISC and TV8 regions were average muscle density, average fat density and related standard deviations, as well as the average soft tissue density and standard deviation of soft tissue density. Muscle area and fat area tissue measurements (mm^2) were also calculated for each of the single-slice scan images. Total CT predicted carcass fat (Pr_Cfat), as a measure of subcutaneous and intermuscular fat in the entire carcass, was also derived using a breed-specific prediction equation from Macfarlane et al (2006b) detailed in chapter 2, section 2.3.2. Details, acronyms and descriptions of each SCTS trait are presented in Table 3.1. CT traits and meat quality traits included in this study which were also in chapter 2 are not described but can be found in chapter 2, Table 2.1.



(a) (b)

Figure 3.1 Detailed tomograms, single slice and spiral images produced during CT scanning

(a) First image where TPLV7 appears *(i),* last image where TPLV1 is no longer visible *(ii)* and 3D rendered stack of selected images *(iii)*

(b) Scan image from ischium region *(i)* and scan image from 8th thoracic vertebra region *(ii)*

Table 3.1: Acronyms and summary statistics of SCTS traits along with trait descriptions, means and standard deviations (SD) in the Texel data utilised in the prediction of intra-muscular fat and shear force (n = 370)

Trait	Acronym	Trait Description	Mean	SD
CT Traits				
	w_md	Average muscle density in the loin spiral scan (weighted by area in each component image) (HU)	46.13	2.22
	w_msd	SD of muscle density in the loin spiral scan (weighted by area in each component image) (HU)	19.91	1.25
	w_fd	Average fat density in the loin spiral scan (weighted by area in each component image) (HU)	-63.97	4.65
	w_fsd	SD of fat density in the loin spiral scan (weighted by area in each component image) (HU)	40.63	3.49
	m_vol	Muscle tissue volume in the loin spiral scan (mm^3)	1,827,238	280,858
	f_vol	Fat tissue volume in the loin spiral scan (mm^3)	298,327	180,327
	w_std	Soft tissue density in the loin spiral scan weighted by area (HU)	31.41	8.43
	w_stsd	SD of soft tissue in the loin spiral scan weighted by area (HU)	42.79	6.17

3.3.3 Slaughter procedure and meat quality parameter measurements

Slaughter procedures and meat quality parameter measurements were entirely the same as described in Chapter 2, section 2.3.3.

3.3.4 Statistical analysis

Before any statistical analyses, lambs included in the combined data set with no IMF data were removed (n = 2), lambs without full CT information were removed (n = 2), and finally lambs with IMF content greater than three standard deviations from the mean were identified as outliers and also removed (n = 3). Initial regression analysis and subsequent model checking (distribution of residuals) suggested the need for transformation of shear force data. As a result, shear force was log-transformed before any regression analysis. The effect of the number of days from CT scanning to slaughter (group 1: 4-8 d; group 2: 32-33 d) on the traits was tested using a general ANOVA in Genstat14^{TM} adjusting for Pr_Cfat, and provided evidence of no significant effect on IMF content (P = 0.80) or shear force (P = 0.07). The term was also fitted as an independent variable in the regression models in order to test the relationship between days to slaughter and the CT variables, and was not significant when tested on IMF (P = 0.71) and shear force (P = 0.19) and therefore was not included in the subsequent analysis. A summary of the CT traits tested in the models are presented in Table 3.1. Histograms of shear force before transformation and IMF are presented in Figure. 3.2.

Sixteen models were tested in the analyses (Table 3.2), termed models A-P, using both information from SCTS only (^{sp}) and a combination of SCTS and two-dimensional, single-slice scan information (^{com}). Models with one or two variables included in the maximum model were analysed using simple and multiple linear regression, respectively, whilst models employing CT data with more than two variables were analysed using stepwise linear regression (Genstat14^{TM}; Payne et al., 2012) to optimize the number and combination of independent variables from the maximum fitted model. Models were then tested for

significant differences between correlation coefficients ($\sqrt{\text{Adj } R^2}$) applying standard methods using Fisher's Z transformation (Rasch et al., 1978). Final models were identified as those with significantly greater prediction accuracies of IMF and shear force than the baseline model (Model A). These models were then cross-validated. During cross-validation, available data were split using a natural time series separation in the data, as described by Snee (1977). The Exp. 1 data (n = 236) was employed as a calibration data set, and Exp. 2 data (n = 134) as a validation data set. Summary statistics for SCTS measured traits for both calibration and validation data sets are presented in Table 3.3. CT traits and meat quality traits included in this study which were also in chapter 2 are not described but can be found in chapter 2, Table 2.4.

The fitted terms identified in the most accurate prediction models derived from the regression analyses using the entire data set were used to produce prediction equations using the calibration data set (Exp. 1). These equations were then used to predict IMF and shear force of the lambs included in the independent validation data set (Exp. 2). The coefficient of determination (R^2) and residual mean square error of prediction (RMSEP) were calculated for the predicted IMF and shear force against chemically extracted IMF and mechanical shear force, to identify the simplest and most reliable single predictive model or group of predictive models. Histograms of IMF and shear force in the calibration and validation data can be found in Figure 3.3.

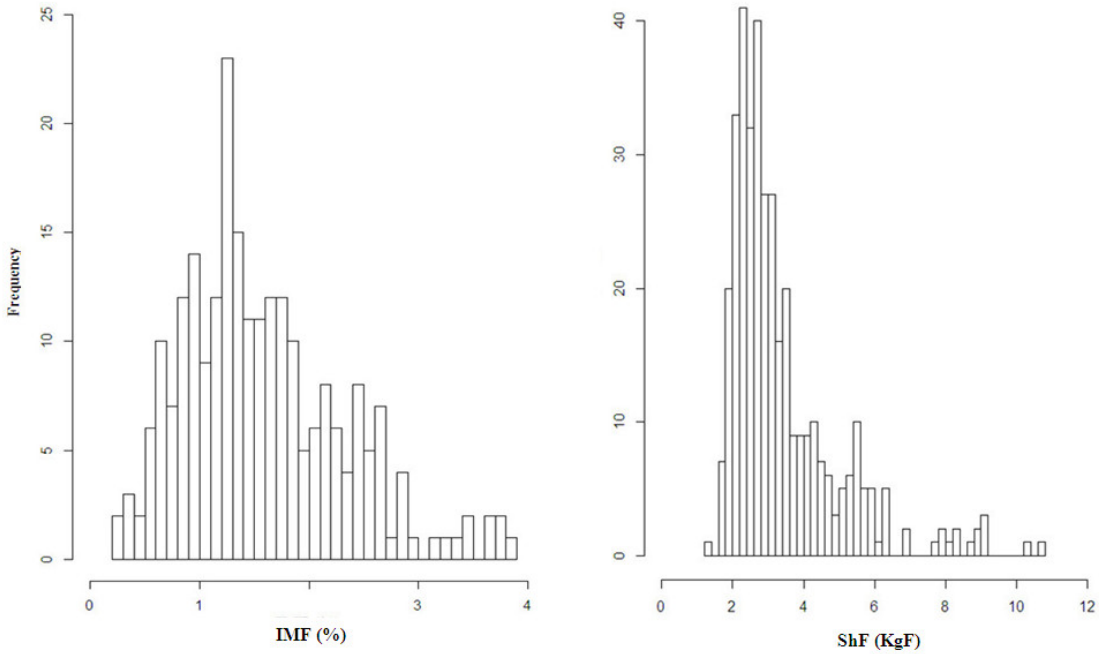


Figure 3.2 Histograms of chemically extracted intramuscular fat percentage (IMF %) and shear force (ShF, kgF) measured in the loin of the Texel lambs (n = 370)

Table 3.2: Terms included in the maximum linear regression models tested before stepwise regression using both spiral CT scan parameters only (sp) and spiral CT scan parameters combined with two dimensional single-slice scan parameters (com).

	Maximum Models	
	SCTS parameters only (sp)	**SCTS + 2D single-slice scan parameters (com)**
A	Pr_Cfat	Pr_Cfat
B	Pr_Cfat, w_md	Pr_Cfat, w_md, ISCMD, TV8MD
C	Pr_Cfat, w_fd	Pr_Cfat, w_fd, ISCFD, TV8FD
D	Pr_Cfat, m_vol	Pr_Cfat, m_vol, ISCMA, TV8MA
E	Pr_Cfat, f_vol	Pr_Cfat, f_vol, ISCFA, TV8FA
F	Pr_Cfat, w_md, w_fd	Pr_Cfat, w_md, w_fd, ISCMD, TV8MD
G	Pr_Cfat, m_vol, f_vol	Pr_Cfat, m_vol, f_vol, ISCMA, TV8MA, ISCFA, TV8FA
H	Pr_Cfat, w_md, w_msd	Pr_Cfat, w_md, w_msd, ISCMD, ISCMSD, TV8MD, TV8MSD
I	Pr_Cfat, w_fd, w_fsd	Pr_Cfat, w_fd, w_fsd, ISCFD, ISCFSD, TV8FD, TV8FSD
J	Pr_Cfat, w_md, w_msd, w_fd, w_fsd	Pr_Cfat, w_md, w_msd, w_fd, w_fsd, ISCMD, ISCMSD, TV8MD, TV8MSD, ISCFD, ISCFSD, TV8FD, TV8FSD
K	Pr_Cfat, w_md, w_msd, w_fd, w_fsd, f_vol	Pr_Cfat, w_md, w_msd, w_fd, w_fsd, f_vol, ISCMD, ISCMSD, TV8MD, TV8MSD, ISCFD, ISCFSD, TV8FD, TV8FSD, ISCFA, TV8FA
L	Pr_Cfat, w_md, w_msd, w_fd, w_fsd, m_vol, f_vol	Pr_Cfat, w_md, w_msd, w_fd, w_fsd, m_vol, f_vol, ISCMD, ISCMSD, TV8MD, TV8MSD, ISCFD, ISCFSD, TV8FD, TV8FSD, ISCMA, ISCFA, TV8MA, TV8FA
M	Pr_Cfat, w_std	Pr_Cfat, w_std, ISCSTD, TV8STD
N	Pr_Cfat, w_std, w_stsd	Pr_Cfat, w_std, w_stsd, ISCSTD, ISCSTSD, TV8STD, TV8STSD
O	Pr_Cfat, w_std, w_stsd, f_vol	Pr_Cfat, w_std, w_stsd, f_vol, ISCSTD, ISCSTSD, TV8STD, TV8STSD, ISCFA, TV8FA
P	Pr_Cfat, w_std, w_stsd, f_vol, m_vol	Pr_Cfat, w_std, w_stsd, f_vol, m_vol, ISCSTD, ISCSTSD, TV8STD, TV8STSD, ISCFA, ISCMA, TV8FA, TV8MA

Explanations of acronyms used in the models can be found in Table 3.1

Table 3.3: Summary statistics of SCTS traits along with trait descriptions, means and standard deviations (SD) in the calibration (n=236) and validation (n=134) data sets

		Calibration Data (n=236)		Validation Data (n=134)	
Trait	**Acronym**	**Mean**	**SD**	**Mean**	**SD**
	w_md	45.98	2.30	46.40	2.05
	w_msd	20.11	1.25	19.55	1.19
	w_fd	-64.36	4.41	-63.29	5.01
	w_fsd	40.37	3.46	41.08	3.52
	m_vol	1,907,573	260,502	1,686,351	259,734
	f_vol	329,122	195,934	244,321	133,477
	w_std	30.35	9.18	33.27	6.55
	w_stsd	43.65	6.57	41.27	5.09

Explanations of acronyms used in the models can be found in Table 3.1

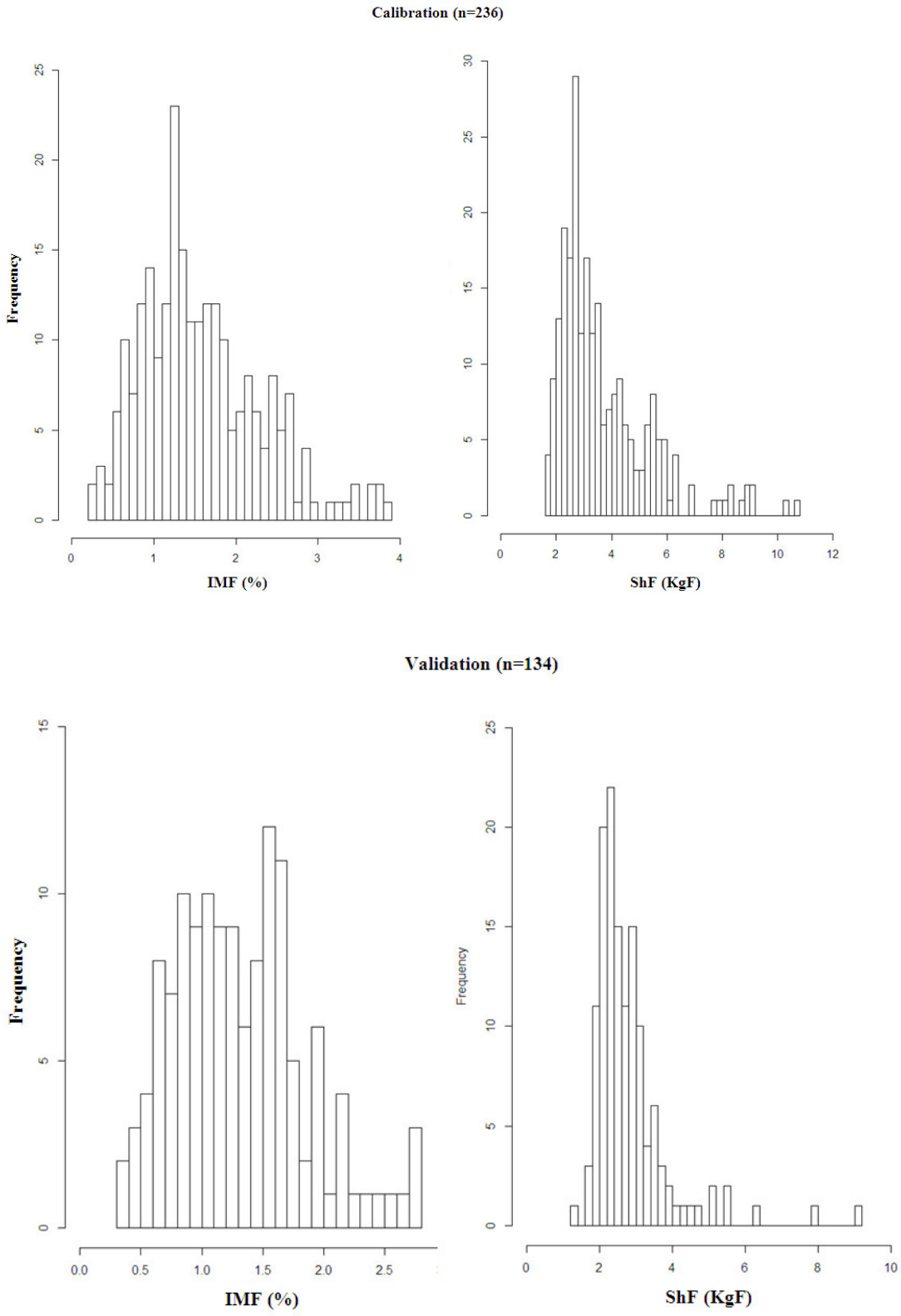


Figure 3.3 Histogram of chemically extracted intramuscular fat percentage (IMF %) and shear force (ShF, kgF) measured in the loin in the calibration and validation data sets

3.4 Results

3.4.1 Predicting shear force and IMF content using SCTS information

Very little of the variation in shear force was accounted for by Pr_Cfat (Adj R^2 = 0.03, RMSE = 0.16), however Pr_Cfat accounted for a moderate amount of the variation in IMF (Adj R^2 = 0.50, RMSE = 0.47). Compared to the baseline (Model A; Table 3.2), which uses information only from CT derived predicted carcass fat, seven models that included additional spiral CT variables, from the fifteen models tested, were identified as being significantly more accurate in the prediction of IMF ($P < 0.05$). None of the models using only spiral CT information (^{sp}) were significantly more accurate ($P > 0.05$) in prediction of shear force when compared to the baseline (Table 3.4).

From the seven models using only SCTS information identified with significantly increased prediction ability of IMF when compared to Model A, the model with the greatest accuracy was identified as model L^{sp} (Adj R^2 = 0.70). This model included CT predicted carcass fat (Pr_Cfat), weighted average muscle density (w_md), fat volume and muscle volume (f_vol, m_vol), resulting in the prediction equation:

$y = 7.773 + 0.1808 \times PrCfat - 0.1379 \times w\_md + 0.000000881 \times f\_vol - 0.0000000338 \times m\_vol.$

The six remaining models including only SCTS information identified as better predictors of IMF than Pr_Cfat alone were compared with model L^{sp}. Only model P^{sp} (Table 3.4) gave significantly reduced accuracy ($P > 0.05$) compared to model L^{sp}. This left six models with correlation coefficients that were not significantly different, essentially meaning that the prediction ability of these six models is statistically similar, thus identifying a group of models that would predict IMF equally using SCTS information. The variables for models K^{sp} and J^{sp} after the stepwise regression procedure were identical and, hence, model K^{sp} was

also dropped. The final selected models were model B^{sp} (Adj R^2 = 0.67), model F^{sp} (Adj R^2 = 0.68), model H^{sp} (Adj R^2 = 0.67), model J^{sp} (Adj R^2 = 0.69) and model L^{sp} (Adj R^2 = 0.70).

Table 3.4: Regression results for the prediction of shear force and IMF, presented are the adjusted coefficient of determination (Adj R2) and residual mean square error (RMSE) using information from SCTS only (sp) or a combination of SCTS and two dimensional single-slice scans (com)

	Shear Force				**IMF**			
	sp		**com**		**sp**		**com**	
Model	**Adj R^2**	**RMSE**	**Adj R^2**	**RMSE**	**Adj R^2**	**RMSE**	**Adj R^2**	**RMSE**
A	0.03	0.16	0.03	0.16	0.50	0.47	0.50	0.47
B	0.03	0.16	0.07	0.16	0.67**	0.39	0.68**	0.39
C	0.04	0.16	0.05	0.16	0.51	0.48	0.52	0.48
D	0.04	0.16	0.04	0.16	0.56	0.46	0.60	0.43
E	0.03	0.16	0.10*	0.16	0.55	0.46	0.58	0.45
F	0.04	0.16	0.09	0.16	0.68**	0.39	0.70**	0.38
G	0.04	0.16	0.10*	0.16	0.58	0.45	0.60	0.43
H	0.04	0.16	0.09	0.16	0.67**	0.39	0.68**	0.39
I	0.05	0.16	0.09	0.16	0.55	0.46	0.56	0.46
J	0.05	0.16	0.12*	0.16	0.69**	0.38	0.70**	0.37
K	0.05	0.16	0.13*	0.15	0.69**	0.38	0.70**	0.37
L	0.06	0.16	0.13*	0.15	0.70**	0.38	0.71**	0.37
M	0.02	0.16	0.08	0.16	0.54	0.47	0.63*	0.42
N	0.02	0.16	0.09	0.16	0.57	0.45	0.66**	0.40
O	0.03	0.16	0.10*	0.16	0.59	0.44	0.67**	0.40
P	0.04	0.16	0.10*	0.16	0.62*	0.42	0.67**	0.39

sp Using SCTS information
com Using a combination of SCTS and single-slice CT information
*Adj R^2 differs significantly from the baseline model (A) (P > 0.05)
**Adj R^2 does not differ significantly from the most accurate model (L) (P < 0.05)

3.4.2 Predicting shear force and IMF content using a combination of SCTS and single-slice scan information

Models using a combination of SCTS information and single-slice scan information (^{com}) were compared to the simple linear model using only Pr_Cfat for the predictions of both shear force and IMF. In the analysis for the prediction of shear force, prediction accuracies

were significantly improved with the inclusion of information from the single-slice scan images (ISC, TV8). Nonetheless, the overall results show that the maximum prediction accuracy achieved for shear force, from models developed was Adj R^2 = 0.13 (Table 3.4).

In the prediction of IMF, ten of the fifteen models tested were significantly greater in prediction accuracies than that of Pr_Cfat alone ($P < 0.05$). From these models the single 'best' model was identified as model L^{com} (Adj R^2 = 0.71):

$y = 7.675 + 0.3125 * PrCfat - 0.0978 \times w\_md + 0.0000000299 \times m\_vol + 0.000001196 \times f\_vol + 0.0168 \times ISCMD + 0.0371 \times ISCMSD - 0.0000393 \times ISCMA - 0.0543 \times TV8MD + 0.0000236 \times TV8MA - 0.0001298 \times TV8FA$.

Descriptions of acronyms used in the models are described in table 3.1.

All models identified as significant previously were then tested against model L^{com} and any that were significantly different in prediction accuracy were discarded ($P > 0.05$), the only model identified was model M^{com} (Adj R^2 = 0.63). These analyses therefore identified nine "best" models with similar prediction abilities: L^{com} (Adj R^2 = 0.71); F^{com}, J^{com} and K^{com} (Adj R^2 = 0.70); B^{com} and H^{com} (Adj R^2 = 0.68); O^{com} and P^{com} (Adj R^2 = 0.67); and N^{com} (Adj R^2 = 0.66). Regression results for all models are presented in Table 3.4.

3.4.3 Model cross-validation and selection

Given the poor prediction abilities of CT for shear force using the parameters tested, cross-validation analysis for the prediction of shear force was not carried out. Fourteen possible models in the prediction of IMF were identified. None of these models had significantly less prediction accuracy ($P > 0.05$) than the single 'best' model from both SCTS information and a combination of SCTS information and single-slice scan information (Model L^{com}), so all were retained for cross-validation analyses. For cross-validation, fourteen prediction equations were derived using the calibration data set (n = 236), corresponding to the independent variables identified in the final selected models from the original stepwise

regression analyses. The models were then used to predict the chemical IMF values of lambs in the independent validation data set (n = 134). Final cross-validation results, coefficients of determination (R^2) and residual mean square errors of prediction (RMSEP) are presented in Table 3.5.

The model with the strongest cross-validity was model N^{com} ($R^2 = 0.67$, RMSEP = 0.40) using both SCTS information and single-slice scan information, including CT predicted carcass fat (Pr_Cfat), weighted average density of soft tissue and its standard deviation (w_std and w_stsd) in the spiral scan of the loin, average soft tissue density and its standard deviation in the ischium scan (ISCSTD and ISCSTSD), average soft tissue density in the 8^{th} thoracic vertebra scan and its standard deviation (TV8STD and TV8STSD). The R^2 of this model (N^{com}, $R^2 = 0.67$) was compared with the thirteen remaining models in the cross-validation analysis using Fisher's z transformation (Rasch et al., 1978). All of the models performed as well as model N in the cross-validation analysis ($P < 0.05$; $R^2 = 0.59$ to 0.66). This left fourteen models for consideration as predictors of IMF, five of which used only SCTS information alongside Pr_Cfat, and nine of which used a combination of SCTS information and single-slice information alongside Pr_Cfat. Details of the final selected prediction models developed from the entire data set are presented in Table 3.6. These included Models B^{sp}, F^{sp}, H^{sp}, J^{sp} and L^{sp} using SCTS information and models B^{com}, F^{com}, H^{com}, J^{com}, K^{com}, L^{com}, N^{com} , O^{com} and P^{com} using a combination of information from both the single-slice scans and SCTS.

Table 3.5: Cross-validation results: adjusted coefficient of determination (Adj R2), residual mean square error (RMSE) of calibration; and coefficient of determination (R2) and residual mean square error of prediction (RMSEP) of the validation data

Model	**Calibration (n=236)**		**Validation (n=134)**	
	Adj R^2	**RMSE**	**R^2**	**RMSEP**
B^{sp}	0.69	0.41	0.60	0.34
F^{sp}	0.70	0.41	0.59	0.34
H^{sp}	0.69	0.41	0.60	0.34
J^{sp}	0.70	0.41	0.62	0.33
L^{sp}	0.71	0.40	0.62	0.33
B^{com}	0.71	0.40	0.64	0.32
F^{com}	0.71	0.40	0.64	0.32
H^{com}	0.70	0.40	0.64	0.32
J^{com}	0.72	0.40	0.66	0.31
K^{com}	0.71	0.40	0.65	0.32
L^{com}	0.72	0.39	0.65	0.32
N^{com}	0.66	0.43	0.67	0.31
O^{com}	0.67	0.43	0.64	0.32
P^{com}	0.67	0.42	0.64	0.32

^{sp} Model uses information from spiral scans only

^{com} Model uses information from a combination of spiral and two dimensional scans

Table 3.6: Final prediction models and equations derived from the whole data set, adjusted coefficient of determination (Adj R2) and residual mean square error of the prediction (RMSEP)

Model	Final prediction model equation	Adj R^2	RMSEP
B^{sp}	y=8.048+0.2508*Pr_Cfat-0.1551*w_md	0.67	0.39
F^{sp}	y=7.897+0.2347*Pr_Cfat-0.1720*w_md-0.01514*w_fd	0.68	0.39
H^{sp}	y=7.10+0.2326*Pr_Cfat-0.1474*w_md+0.0319*w_msd	0.67	0.39
J^{sp}	y=7.62+0.1134*Pr_Cfat-0.1566*w_md+0.0401*w_mSD-0.02682*w_fd-0.0417*w_fsd	0.69	0.38
L^{sp}	y=7.773+0.1808*Pr_Cfat-0.1379*w_md+0.000000881*f_vol-0.000000038*m_vol	0.70	0.38
B^{com}	y=8.275+0.2248*Pr_Cfat-0.1113*w_md-0.0490*TV8MD	0.68	0.39
F^{com}	y=7.794+0.1704*Pr_Cfat-0.1347*w_md-0.01553*w_fd+0.0183*ISCMD-0.0600*TV8MD-0.00471*TV8FD	0.70	0.38
H^{com}	y=7.39+0.2079*Pr_Cfat-0.1043*w_md+0.0298*w_mSD-0.0488*TV8MD	0.68	0.39
J^{com}	y=6.66+0.1054*Pr_Cfat-0.1138*w_md+0.0661*w_mSD-0.02761*w_fd-0.0250*w_fSD-0.0502*TV8MD	0.70	0.37
K^{com}	y=5.78-0.1051*w_md+0.0549*w_mSD-0.01753*w_fd+0.000000769*f_vol+0.0437*ISCMSD-0.00703*ISCFD-0.0189*ISCFSD-0.0533*TV8MD	0.70	0.37
L^{com}	y=7.675+0.3125*Pr_Cfat-0.0978*w_md-0.000000299*m_vol+0.000001196*f_vol+0.0168*ISCMD+0.0371*ISCMSD-0.0000393*ISCMA-0.0543*TV8MD+0.0000236*TV8MA-0.0001298*TV8FA	0.71	0.37
N^{com}	y=7.099+0.1101*Pr_Cfat-0.0305*w_std-0.0368*w_stSD-0.0205*ISCSTD-0.04523*TV8STD+0.0103*ISCSTSD-0.0404*TV8STSD	0.66	0.40
O^{com}	y=7.382+0.2253*Pr_Cfat-0.0251*w_std-0.0332*w_stSD+0.000001035*f_vol-0.0322*ISCSTD+0.0142*ISCSTSD-0.04967*TV8STD-0.0387*TV8STSD-0.0001178*ISCFA-0.0001394*TV8FA	0.67	0.40
P^{com}	y=8.554+0.4879*Pr_Cfat-0.0330*w_std-0.0448*w_stSD+0.000001051*f_vol-0.000000243*m_vol-0.0000566*ISCMA-0.05713*TV8STD-0.0357*TV8STSD-0.0002859*TV8FA+0.0000371*TV8MA	0.67	0.39

^{sp} Model uses information from Pr_Cfat and spiral scans only
^{com} Model uses information from Pr_Cfat and a combination of spiral and single-slice scans
Explanations of acronyms used in the models can be found in Table 3.1

3.5 Discussion

It has been demonstrated in previous studies that information from single or multiple CT scans can provide moderately accurate predictions of IMF in different sheep breeds. Prediction accuracies range from R^2 = 0.33 to 0.68. Karamichou et al. (2006) reported a

prediction accuracy for Scottish Blackface lambs of R^2 = 0.33, using only information on muscle density provided by three single-slice scan images. Similar prediction accuracies (R^2 = 0.36) were reported for purebred Texel lambs by Lambe et al. (2010a), using muscle density measurements from one single scan at the 5th lumbar vertebra. In the same study, these accuracies were improved by including fat density and the standard deviation of both fat and muscle density to the model, resulting in an increase in accuracy of 12% (R^2 = 0.48). Similar lean tissue measurements, as well as a fat tissue area measurements (mm^2) from a single cross-sectional scan at the 2nd lumbar vertebra, were used to predict IMF in a study including three terminal sire breeds (Suffolk, Texel and Charollais), achieving a prediction accuracy of 57% (Macfarlane et al., 2005). These studies and the work carried out in chapter 2 , reporting a maximum prediction accuracy of 68%, have provided evidence of the potential use of single-slice CT scanning as a predictor of IMF in different sheep breeds.

The results from this study provide evidence that slight further improvements in the prediction of IMF are possible and the use of information from both spiral CT scans and a combination of SCTS and single-slice scans can adequately predict intramuscular fat content in the loin of purebred Texel sheep. Prediction models using SCTS parameters for the assessment of IMF content, achieved a maximum accuracy of AdjR^2 = 0.70 and 0.71, using either SCTS information only, or a combination of SCTS and single-slice scan information respectively.

The results from this study indicate that there are several potential prediction models that may be developed, using different combinations of CT and SCTS parameters. A number of potential prediction models, with increasing degrees of complexity, had similar prediction accuracies for IMF, which again could be indicative of a possible ceiling in the achievable prediction accuracies we may expect using these types of CT variables, as discussed in chapter 2 (Max. R^2 = 0.68). It is also apparent that the application of this relatively novel

SCTS technology does not result in significant gains in accuracy when compared to the accuracies reported in chapter 2 ($P < 0.05$).

Models that included increasing numbers of independent variables appeared to be slightly less transferable when cross-validated against the independent time series data. Although not significant, the models including fewer independent variables and more direct measures of soft tissue density (average and standard deviation) were generally more robust during cross-validation. This suggests that the complexity of the model may have an effect on the accuracy of prediction when applied to an independent data set, as discussed in chapter 2 where, in previous studies, the complexity of models used to predict IMF in pork loins *in vivo* and *post-mortem* had an effect on the transferability of the models to independent data sets (Font-i-Furnols et al., 2013; Kongsro and Gjerlaug-Enger, 2013).

As was reported in chapter 2, for single-slice CT, the use of SCTS parameters failed to adequately estimate shear force of the loin, producing an upper limit of $R^2 = 0.13$. Similar studies carried out by Lambe et al. (2008c) and Karamichou et al. (2006) reported low phenotypic correlations between two dimensional CT variables and shear force ($r = 0.15 – 0.22$, $r = 0.16$ respectively).

As previously discussed in chapter 2, IMF is regarded as an important factor in the eating quality of meat when related to mouth feel, tenderness, juiciness and species-specific flavour, the relationship between shear force and IMF is less clear. Other factors such as cooking loss, ultimate pH, post-mortem glycolysis and conditioning (ageing) play an important role in the conversion of muscle to meat, and these *post-mortem* changes, may have significant effects on shear force results and are therefore not picked up by CT technology prior to slaughter. There is evidence of a linear relationship between shear force values in cooked meat samples and solvent-extracted IMF content in raw meat samples and

it is generally accepted that this relationship exists (Hopkins et al., 2006; Pannier et al., 2014b; Safari et al., 2001), although the size of the effect is often debated.

This study shows that there may be several models and combinations of parameters using SCTS technology to predict IMF as a proxy for meat eating quality traits. This being the case, it is clear that the application of CT predicted traits such as IMF could be included into current breeding programmes to allow for the simultaneous selection of animals with desirable production, carcass and meat quality traits. Although CT measurements are initially expensive to record, however, CT scanning is now routinely incorporated into Terminal sire breeding programmes in the UK. And as part of this scanning, 3-dimensional information is routinely captured, therefore such novel traits (including the use of SCTS) can be retrospectively estimated on a large numbers of animals and incorporated into current breeding programmes with no extra scanning costs associated.

Current 'two stage' selection practices for carcass composition select in the first step animals using ultrasound scanning, which is more applicable to a larger number of animals, providing cost-effective, but less accurate, measures of carcass traits. This is then followed by further selection of the top ranked animals from the pre-selection stage, using more accurate measurements of carcass traits with CT (Bunger et al., 2011).

This current research and the study in chapter 2 alongside further research into the inclusion of meat quality traits into breeding programmes, would allow further selection of the top ranked animals from the pre-selection stage based on more accurate measurements of carcass traits alongside IMF. The application of these methods across different terminal sire breeds, and the investigation of genetic relationships between CT predictors and other relevant carcass traits, could enable IMF predictors to be included in current breeding programmes, providing valuable information related to meat eating quality aspects of lamb.

3.6 Conclusion

In conclusion, the prediction of mechanical shear force could not be achieved at an acceptable level of accuracy employing information from SCTS. However, the prediction of IMF in the loin employing information from SCTS with or without additional information from single-slice scans was more promising. This study provides valuable evidence that the prediction of IMF, as a proxy measure of related meat eating quality traits, for Texel lambs *in vivo* can be achieved using spiral x-ray CT technology. However the increase in accuracy when employing SCTS technology was not significant when compared to previous studies using single slice scanning procedures ($P < 0.05$; Chapter 2). This suggests that the use of SCTS technology in the prediction of IMF does not adequately increase prediction accuracies to justify additional image analysis involved in the processing of the resulting data. Therefore we can conclude that, although the methods used in this study were successful in the prediction of IMF, the increased image analysis and processing currently required does not justify the increase in accuracy achieved when compared to CT predictors that could be measured using current single-slice scan procedures.

Chapter 4: Comparison of carcass and meat quality traits of divergent sheep genotypes and *In vivo* prediction of intramuscular fat content in the loins of divergent sheep genotypes using X-ray computed tomography

4.1 Summary

The purpose of this chapter was firstly to compare different genotypes regarding carcass fatness and IMF content, and secondly to investigate the application and accuracy of CT-derived IMF estimations detailed in chapter 2, developed in terminal sires, on divergent commercial lamb genotypes. Data were available from three different genotypes: Texel (n = 370), Scottish Blackface (n = 230) and Texel cross Scotch Mule (n = 165). Lambs were CT scanned pre-slaughter and post-slaughter IMF was measured in the dissected loin muscle (M. Longissimus lumborum). After adjusting for pre-slaughter live weight, IMF was significantly affected by genotype (P<0.001) with Scottish Blackface lambs having higher levels of IMF when compared to Texel cross mule lambs, and the lowest levels of IMF were in the purebred Texels at the same liveweight or at similar levels of carcass fatness . Within genotype, females had significantly higher levels of IMF in both the purebred Texels and Scottish Blackface lambs, when compared at similar levels of carcass fat and liveweight (P<0.05) . In the estimation of IMF content from CT data, using models previously developed from terminal sire data in chapter 2 (Purebred Texel), accuracies were within the range R^2 = 0.57 – 0.64 in Scottish Blackface lambs and R^2 = 0.37 – 0.38 in Texel cross Scotch Mule lambs. The results provide evidence that the models developed on terminal sire data to predict IMF using CT are transferable across divergent genotypes, producing acceptable accuracies given that there is currently no non-destructive method for predicting this important meat quality trait (IMF) in-vivo.

4.2 Introduction

X-ray computed tomography (CT) not only provides information on carcass tissue areas, volumes and weights, but resulting CT muscle density parameters have also been shown to

be good predictors of IMF (Karamichou et al., 2006; Lambe et al., 2010a; Macfarlane et al., 2005).

Current studies have concentrated on the application of CT information in the prediction of IMF levels in the loin of terminal sire breeds, producing prediction accuracies ranging from 63% - 71% (Chapter 2 and 3), employing information from various CT variables collected during routine single-slice and spiral CT scanning procedures (Bunger et al., 2011).

However, CT also has the potential to be of interest in the acceleration of genetic improvement of carcass traits in maternal and crossbreeding selection programmes (Conington et al., 2006), because of its higher accuracy compared to the sole use of ultrasound techniques. Crossbred lambs make up the majority of the UK slaughter population and about 75% of all ewe mating's in 2012 were crosses (either purebred and mated to a sire from another breed type or a crossbred ewe type mated to a sire of another breed type). It was also estimated that 60% of the slaughter lamb crop in the UK was born to ewes of hill or hill cross breed types (Pollott, 2012). The purpose of this chapter was (i) to compare different genotypes regarding their IMF levels and (ii) to investigate the accuracy of prediction equations to estimate IMF *in-vivo* using CT data, that were developed using data from a terminal sire sheep breed (pure-bred Texel; Tex), when used in two divergent genotypes: a typical UK commercial crossbred slaughter lamb (Texel x Scottish Mule; TexX) and a UK hill sheep breed (Scottish Blackface; SBF).

4.3 Materials and methods

4.3.1 Experimental Animals

The Texel lambs are described in Chapter 2, however to act as a brief reminder and also to compare alongside the other breeds used in this study, they are also presented here. Data from Tex lambs (n=377) were collected in two previous studies, for which full details can be

found in Lambe *et al.* (2008) and Lambe *et al.* (2011) and Chapter 2. The combined studies provided data comprising of information from Texel lambs of both sexes (female and entire males) produced over three separate years (2003, 2004 and 2009). Lambs were reared to weaning as either singles (n=184), or twins (n=168), or were artificially hand reared (n=25).

Data from SBF (n=233) lambs of both sexes (females and entire males) were collected as part of the same trial as for the Texels in 2003 and 2004, full materials and methods can be found in Lambe *et al.* (2008). Lambs were reared to weaning as either singles (n=106), twins (n=124), or artificially hand reared (n=3), and comprised of entire males and females.

Data from TexX lambs (n=168) of both sexes (females and castrates) were collected as part of a separate historical trial conducted in 2006, full materials and methods of which can be found in Lambe *et al* (2010c). Lambs were reared as either twins (n=137) or singles (n=29). Litter size during rearing was unknown for the remaining 2 lambs in the dataset. Details of animals used in the study, including within genotype statistics of sex, live weight, slaughter weight CT carcass fat weight and IMF% can be found in Table 4.1

All lambs in each study were grazed on low-ground pastures, with the Tex and SBF lambs included in the 2003-2004 trial finished with condition score and live weight used as indicators of readiness for slaughter. The remaining Tex lambs (2009) and TexX lambs (2006) were reared to approximately 20 weeks of age, weaned from their mothers, and slaughtered. Measured CT traits, liveweight at CT, and slaughter traits (chemically-extracted IMF and age at slaughter) and their acronyms can be found in Table 4.2.

Table 4.1: Trait descriptions, means and standard deviations (SD) in Purebred Texel (Tex), Scottish Blackface (SBF) and Texel cross Mule (TexX) lambs, within sex

		Tex Male(n=171) Female(n=199)			**SBF** Male(n=127) Female(n=103)			**TexX** Male(n=82) Female(n=83)		
	Trait Description	Mean	Min-max	SD	Mean	Min-max	SD	Mean	Min-max	SD
CTLWT	Live weight at time of CT scanning									
	Male (Castrates in TexX)	36.2	20.6-49	5.18	35.3	29.8-43.6	3.23	40.3	28.6-51.6	4.88
	Female	34.6	22.3-45.1	4.45	33.2	28.1-38.5	2.62	38.9	23.8-49.8	4.33
CT_Age	Age at CT									
	Male (Castrates in TexX)	131	93-202	20.5	141	105-202	22.9	144	132-151	4.3
	Female	133	95-196	21.43	149	109-202	24.3	143	133-152	4.9
Pr_Cfat	CT Predicted total carcass fat weight (kg)									
	Male (Castrates in TexX)	2.1	0-4.8	0.95	2.7	1.2-5.9	0.92	3.3	0.8-7.1	1.16
	Female	2.6	0.3-6.9	1.20	3.3	1.6-5.8	1.01	3.8	0.4-7.3	1.2
Pr_IMF_A	*M. longissimus lumborum* CT predicted extracted intra-muscular fat (%)									
	Male (Castrates in TexX)	1.31	0.2-2.7	0.49	2.0	1.1-2.9	0.38	1.9	0.7-2.9	0.48
	Female	1.63	0.04-3.3	0.57	2.4	1.4-3.5	0.43	2.2	0.4-3.5	0.47
Pr_IMF_B	*M. longissimus lumborum* CT predicted extracted intra-muscular fat (%)									
	Male (Castrates in TexX)	1.25	0.2-2.5	0.42	2.3	1.0-4.2	0.58	2.1	0.9-4.4	0.61
	Female	1.68	0.1-4.1	0.59	3.1	1.7-4.9	0.75	2.5	0.7-4.8	0.69
Chem_IMF	*M. longissimus lumborum* chemically extracted intra-muscular fat (%)									
	Male (Castrates in TexX)	1.25	0.3-3.7	0.59	2.1	0.2-4.4	0.79	2.1	0.8-3.9	0.62
	Female	1.68	0.4-3.9	0.70	2.5	0.4-4.6	0.79	2.2	0.7-3.8	0.59
SL_Age	Age at slaughter									
	Male (Castrates in TexX)	149	109-234	22.8	158	114-229	25.7	149	139-156	4.15
	Female	150	99-228	23.8	168	113-230	27.9	149	139-157	4.9

Table 4.2: Trait descriptions, means and standard deviations (SD) in Purebred Texel (Tex), Scottish Blackface (SBF) and Texel cross Mule (TexX) lambs

		Tex (n=370)			**SBF** (n=230)			**TexX** (n=165)		
	Trait Description	Mean	Min-max	SD	Mean	Min-max	SD	Mean	Min-max	SD
CT Traits										
CTLWT	Live weight at time of CT scanning	35.35	20.6-49	4.87	34.36	28.1-43.6	3.14	39.62	23.8-51.6	4.64
CT_Age	Age at CT	133	93-202	21.01	145	105-202	23.86	144	132-152	4.62
LV5MD	Average muscle density in 2D scan at the 5th lumbar vertebra (HU)	48.30	41.8-55.9	2.65	44.68	38.7-50.3	2.11	46.45	41.2-53.2	2.06
TV8MD	Average muscle density in 2D scan at the 8th thoracic vertebra (HU)	44.68	36.5-54.7	2.98	39.90	32.2-51.1	2.53	41.99	37.3-51.4	2.37
LV5STD	Average soft tissue density in 2D scan at the 5th lumbar vertebra (HU)	36.22	-1.6-49.5	8.09	18.91	-14.4-44.6	12.27	22.62	-15.6-46.5	11.14
TV8STD	Average soft tissue density in 2D scan at the 8th thoracic vertebra (HU)	21.84	-21.1-46.2	11.35	2.54	-26.6-33.9	12.07	5.41	-27.7-34.4	12.34
ISCSTSD	SD of soft tissue density in 2D scan at the ischium (HU)	40.34	29.3-57.9	5.66	49.40	33.9-66.4	6.02	49.04	34.8-60.9	5.58
LV5STSD	SD of soft tissue density in 2D scan at the 5th lumbar vertebra (HU)	40.33	30.4-64.7	6.19	51.46	31.3-69.1	8.09	51.27	-15.6-46.5	8.44
TV8STSD	SD of soft tissue density in 2D scan at the 8th thoracic vertebra (HU)	50.56	34.1-68.1	6.70	58.01	41.6-68.8	5.49	59.34	42.5-71.9	6.40
Pr_Cfat	CT Predicted total carcass fat weight (kg)	2.34	0-6.9	1.11	3.01	1.2-5.9	1.00	3.54	0.4-7.3	1.21
Pr_IMF_A	*M. longissimus lumborum* CT predicted extracted intra-muscular fat (%)	1.48	0.04-3.3	0.56	2.19	1.1-3.5	0.44	2.07	0.4-3.5	0.48
Pr_IMF_B	*M. longissimus lumborum* CT predicted extracted intra-muscular fat (%)	1.48	0.1-4.1	0.56	2.64	1-4.9	0.77	2.29	0.7-4.8	0.68
Slaughter Traits										
Chem_IMF	*M. longissimus lumborum* chemically extracted intra-muscular fat (%)	1.48	0.3-3.9	0.68	2.28	0.2-4.6	0.82	2.14	0.7-3.9	0.61
SL_Age	Age at slaughter	150	99-234	23.3	163	113-230	27.16	149	139-157	4.56

4.3.2 Computed Tomography (CT) measurements

In each of the studies described above lambs were CT scanned prior to slaughter. The CT and image analysis procedures are explained in detail in chapter 2. In brief for the 3 different genotypes two dimensional (2D) cross-sectional scans (Field of view = 450mm, Resolution = 512x512 pixels) were taken at 3 defined anatomical positions, at the ischium bone (ISC), the fifth lumbar vertebra (LV5), and at the 8th thoracic vertebra (TV8) (Figure 4.1). Image analyses were performed to separate carcass from non-carcass tissues (Glasbey and Young, 2002) and the density of each pixel (0.77mm^2) in the carcass portion was allocated to fat, muscle or bone, according to density thresholds using Sheep Tomogram Analysis Routines (STAR) software (Mann et al., 2013). Areas (mm^2) and average densities (Hounsfield units; HU) of each tissue in each 2D image were calculated, as well as standard deviations for the density values of all pixels allocated to each tissue. A novel average soft tissue density (and its standard deviation) was also calculated, combining the information from all pixels allocated as fat or muscle.

CT-predicted carcass fat (Pr_Cfat), as a measure of subcutaneous and intermuscular fat, was also calculated, by combining data from these 3 single-slice CT scans with live weight, using breed-specific prediction equations developed from previous research (Lambe et al., 2006; Macfarlane et al., 2006a; Macfarlane et al., 2006b).

The statistical description for CT traits included in the prediction models as well as the age, live weight at CT scanning and age at slaughter for the breed/crossbred lambs is presented in Table 4.2.

4.3.3 Slaughter and Meat quality measurements

Chemically-extracted IMF (Chem_IMF) in all studies was measured at the University of Bristol post-slaughter in a cross-sectional slice taken from the cranial end of the *M. longissimus lumborum*. Each sample was blended to a fine paste and petroleum ether (B.P.

40-60°C) used as the solvent in a modified Soxhlet extraction (AOAC, 1990). Two tests were performed per sample and averaged.

Results from the Texel study in chapter 2 identified two (A, B) optimum prediction equations based on available CT information with prediction accuracies of Adj R^2 = 0.66 and 0.68 respectively:

A: CT Predicted IMF (Pr_IMF_A) (%) = 6.920 + (Pr_Cfat*0.2425) – (LV5MD*0.0654) – (TV8MD*0.0637)

B: CT Predicted IMF (Pr_IMF_B) (%) = 7.320 + (Pr_Cfat*0.0565) – (LV5STD*0.0626) – (TV8STD*0.03585) + (ISCSTSD*0.02209) – (LV5STSD*0.0565) – (TV8STSD*0.0303)

Where MD = average muscle density, STD = average soft tissue density, STSD = standard deviation of soft tissue density

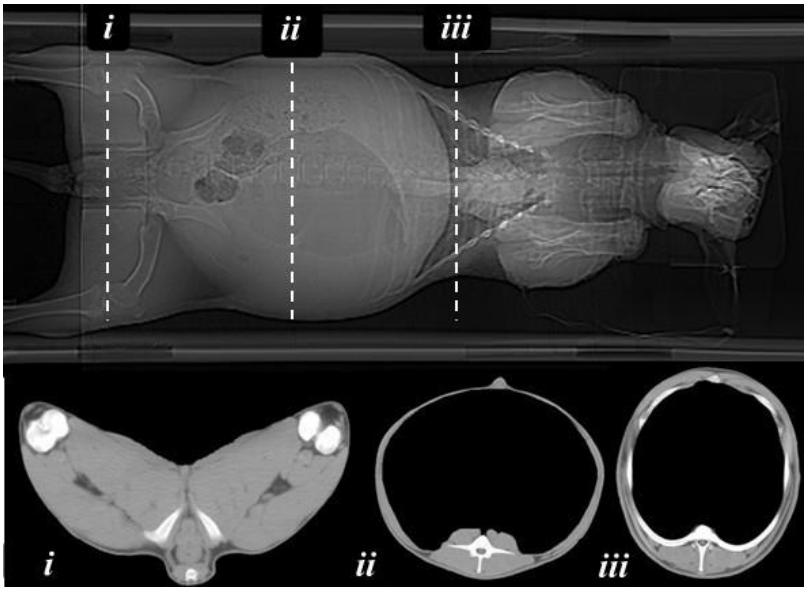


Figure 4.1 Topogram (Top) and single slice CT scan images (bottom) at the ischium (i), 5th lumbar vertebra (ii) and 8th thoracic vertebra (iii)

4.3.4 Statistical analyses

Prior to any statistical analyses, animals with missing values for CT or MQ information were excluded (Tex; n=4, SBF; n=3, TexX; n=2), animals with IMF values greater than three

standard deviations from the mean were considered outliers and also excluded (Tex; n=3, TexX; n=1).

Equations used here for the prediction of IMF using CT variables (Pr_IMF) were derived and validated in chapter 2, using various CT variables from the 3 single-slice CT scans described above in pure-bred Texel lambs (n=370) and relating them to Chem_IMF. Full details of materials and methods can be found in chapter 2.

Assuming that there is no significant effect of experiment or year of trial, a two way general linear model analysis of variance (ANOVA) for unbalanced design in Genstat14^{TM} (Payne et al., 2012) was carried out to estimate the effect of genotype and sex within genotype on Chem_IMF and Pr_Cfat. Analysis of sex across genotype was not carried out as a result of the TexX data including male castrates and the Tex and SBF data including entire males. The initial model for Chem_IMF included live weight at pre-slaughter CT (CTLWT) fitted as a covariate with fixed effects of genotype, and the interaction between genotype and sex. The secondary model for Chem_IMF included CT predicted carcass fat (Pr_Cfat) fitted as a covariate, and fixed effects of genotype, and the interaction between genotype and sex. The model for Pr_Cfat included CTLWT fitted as a covariate with fixed effects for genotype, and the interaction between genotype and sex. Both age at CT scanning (CT_Age) and age at slaughter (SL_Age) effects were tested as covariates in separate models and were shown not to be significant (P = 0.92 and 0.89 respectively).

In the evaluation of prediction models (A and B) across genotypes, models were tested within genotype fitting each model's predicted values as the explanatory variable in a linear regression of Chem_IMF using Genstat14^{TM}. Accuracies for the predictions in SBF and TexX were then compared, using their correlation coefficient (r) applying Fisher's Z transformation (Rasch et al., 1978), against that of the original correlation coefficient (r, with $r = \sqrt{R^2}$) achieved in the terminal sire development data with which these equations were derived.

4.4 Results

4.4.1 Genotype comparison of Chem_IMF and Pr_Cfat

After adjusting for CTLWT, Chem_IMF in the loin was significantly affected by genotype (P <0.001), with the highest levels in SBF, followed by TexX, then Tex (Table 4.3). Sex within genotype was also shown to have a significant effect on Chem_IMF, after adjusting for CTLWT, with females showing higher values than males.

Fitting the same model, but adjusting for Pr_Cfat rather than CTLWT, means for Chem_IMF still showed a significant genotype effect (P <0.001) and each genotype ranked similarly as with the previous model. Sex within genotype for both Tex and SBF was significant, however no significant effect of sex within genotype was shown in the TexX, where the males had been castrated (Genotype x Sex; Table 4.3).

After adjusting for CTLWT, the predicted means for Pr_Cfat show that there was a significant genotype effect on Pr_Cfat (P <0.001), with SBF lambs ranking highest and Tex lambs ranking lowest. A significant sex effect was shown within genotype where females ranked significantly higher than males in all genotypes (P <0.05; Table 4.3)

Table 4.3: Adjusted least square means for the effects of genotype and genotype by sex interaction on Chem_IMF and Pr_Cfat. Standard error of the means (s.e) or standard errors of the difference between means (s.e.d) are shown.

Factor	Genotype					Genotype × Sex					
	Tex	SBF	TexX	s.e.d	*p* value	Tex		SBF		TexX	
						Male (s.e)	Female (s.e)	Male (s.e)	Female (s.e)	Male (s.e)	Female (s.e)
Chem_IMF*	1.51[a]	2.42[b]	1.90[c]	0.06	<0.001	1.23[a] (0.05)	1.77[b] (0.04)	2.12[a] (0.06)	2.72[b] (0.06)	1.81[a] (0.07)	1.99[b] (0.07)
Chem_IMF**	1.67[a]	2.20[b]	1.83[c]	0.05	<0.001	1.55[a] (0.04)	1.78[b] (0.04)	2.10[a] (0.05)	2.30[b] (0.05)	1.91[a] (0.06)	1.76[a] (0.06)
Pr_Cfat	2.44[a]	3.39[b]	2.81[c]	0.06	<0.001	2.04[a] (0.05)	2.83[b] (0.05)	2.87[a] (0.06)	3.89[b] (0.06)	2.39[a] (0.07)	3.22[b] (0.07)

*Model corrected for CTLW, **Model corrected for Pr_Cfat, Genotype means not sharing a common character in their superscript, within factor (same row), are significantly different (p<0.05), Genotype × Sex means not sharing a common character in their superscript, within genotype (same column) and within factor (same row), are significantly different (p<0.05)

Table 4.4: Validation of selected models across SBF and TexX data sets

	Texel	SBF	TexX
Model	Adj R^2 (RMSEP)	R^2 (RMSEP)	R^2 (RMSEP)
A	0.66 (0.40)	0.64 (0.49)	0.37* (0.48)
B	0.68 (0.39)	0.57* (0.54)	0.36* (0.49)

* Coefficient of determination (R^2) is significantly different from development data (Texel) (P<0.05)

4.4.2 Accuracy of prediction equations in SBF and TexX

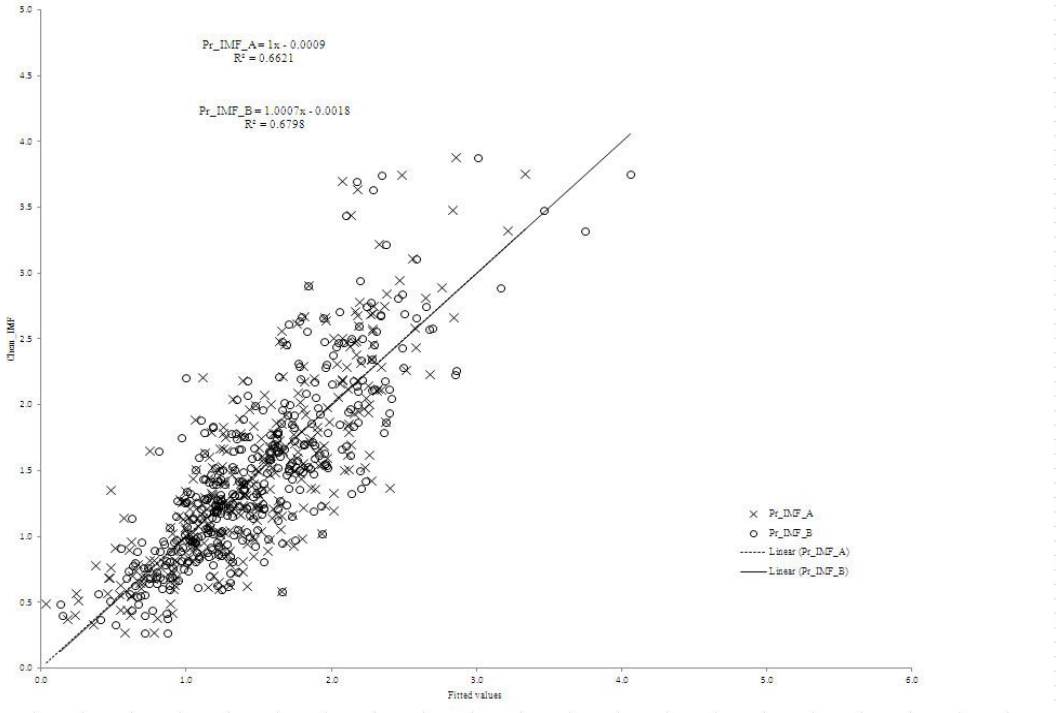
Model A, derived using Tex data (Chapter 2), which included information from CT predicted carcass fat (Pr_Cfat), average muscle density in the fifth lumbar vertebra scan (LV5MD) and average muscle density in the eighth thoracic vertebra scan (TV8MD), performed well when validated in the SBF data, resulting in $R^2 = 0.64$, but resulted in a significant reduction in the coefficient of determination (R^2) when validated using the TexX data ($R^2 = 0.37$; Table 4.4). Model B, also derived on Tex data, which included information from CT predicted carcass fat (Pr_Cfat), average soft tissue density in the fifth lumbar vertebra and eighth thoracic vertebra scans (LV5STD, TV8STD) and the standard deviation of soft tissue density in the ischium, fifth lumbar vertebra and eighth thoracic vertebra scans (ISCSTSD, LV5STSD, TV8STSD), explained a high proportion of the variance ($R^2= 0.68$) in the training data set (Tex), but explained significantly less variance when validated against both the SBF data and the TexX data ($R^2 = 0.57$ and 0.36 respectively, Table 4.4). Plots of the fitted values from both models (A and B) for all three data sets can be seen in Figure 4.2.

Because the Tex data were used to derive the prediction equations, the regression coefficients for both models are close to 1, as expected (Figure 4.2i). The slopes in Figure 4.2ii (SBF data) diverge from unity, model A; $b = 1.49$, $P < 0.0001$ and model B; $b = 0.81$, $P < 0.0001$. Both models produce a bias, with model A overestimating lower values and underestimating larger values, whilst model B overestimates across the range of values, with that overestimation increasing as values increase. The slopes in Figure 4.2iii (TexX data) also diverge from unity, model A; $b = 0.78$, $P = 0.004$ and model B; $b = 0.53$, $P < 0.0001$. Both models produce a bias with both model A and B underestimating lower values and overestimating higher values, however the bias appears to be greater in model B.

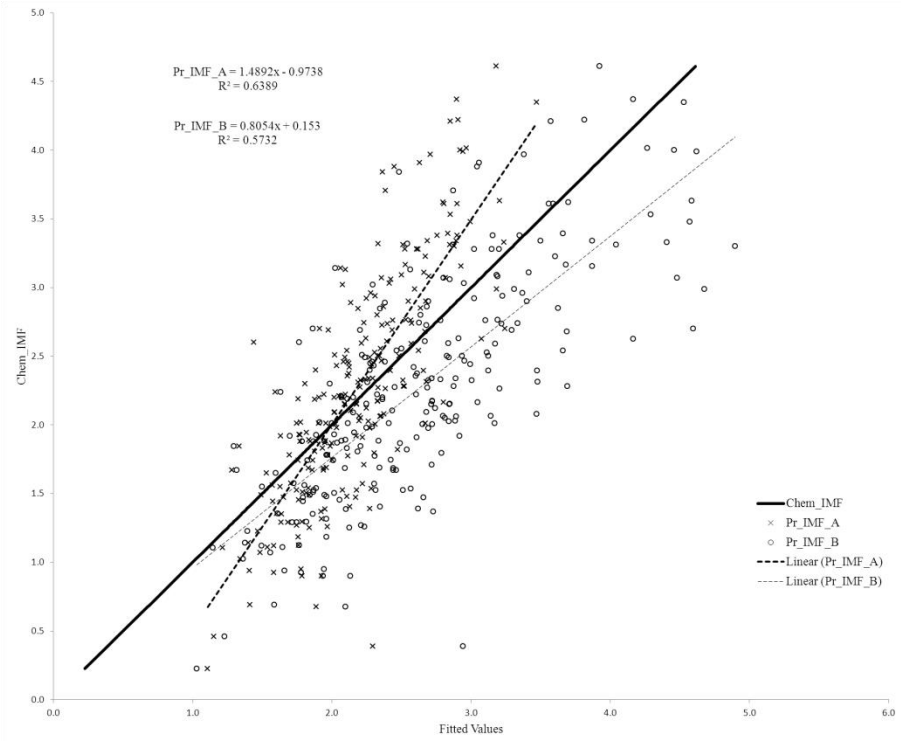
Plots of the residuals against Chem_IMF from both models (A and B) for all three data sets can be seen in Figure 4.3. The slopes for the residuals in the Tex data (Figure 4.3i) indicate

that both models are overestimating smaller values and underestimating larger values and indicate a bias in the slope in both model A and B (b = -0.34, P<0.001 and b = -0.32, P<0.001 respectively). The slopes in Figure 4.3ii (SBF) indicate that both models overestimate smaller values and underestimating larger values and again indicate a bias in both model A and B (b = -0.57, P<0.001 and b = -0.29, P <0.001 respectively).The slopes for the residuals in the TexX data (Figure 4.3iii) indicate that both models are overestimating smaller values and underestimating larger values and once again indicate a bias in the models (A; b = -0.52, P<0.001 and B; b = -0.33, P<0.001).

(i)



(ii)



(iii)

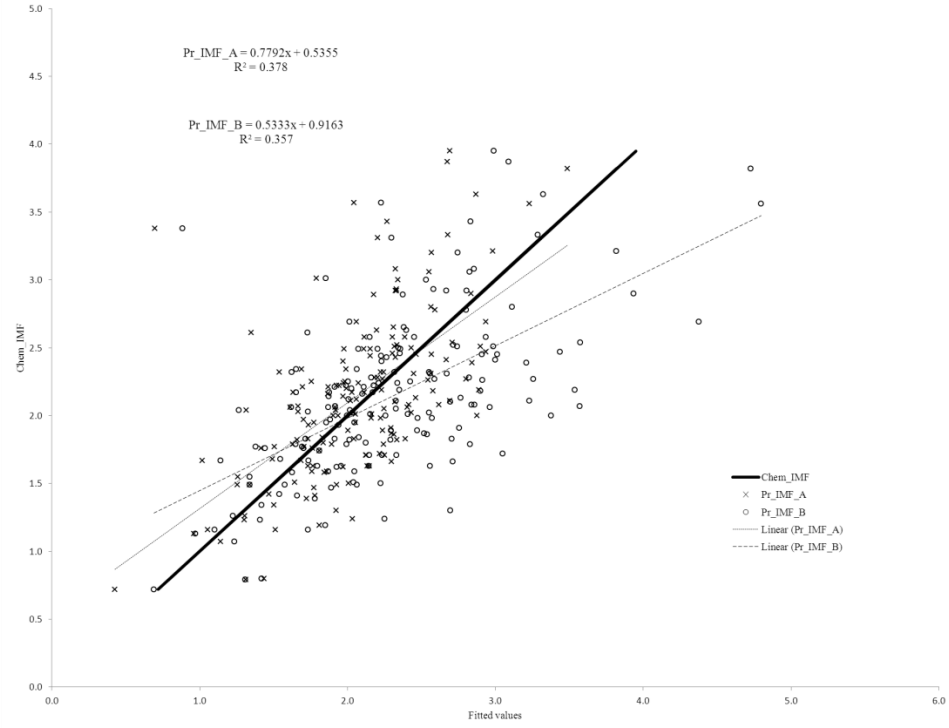
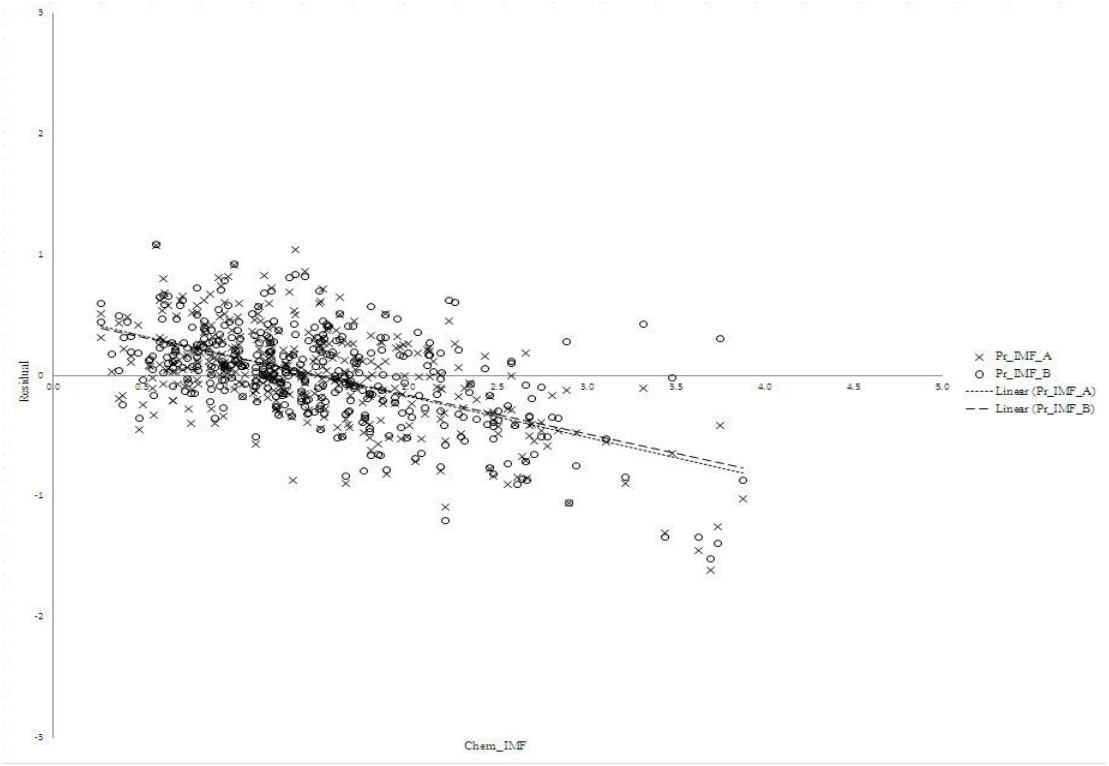
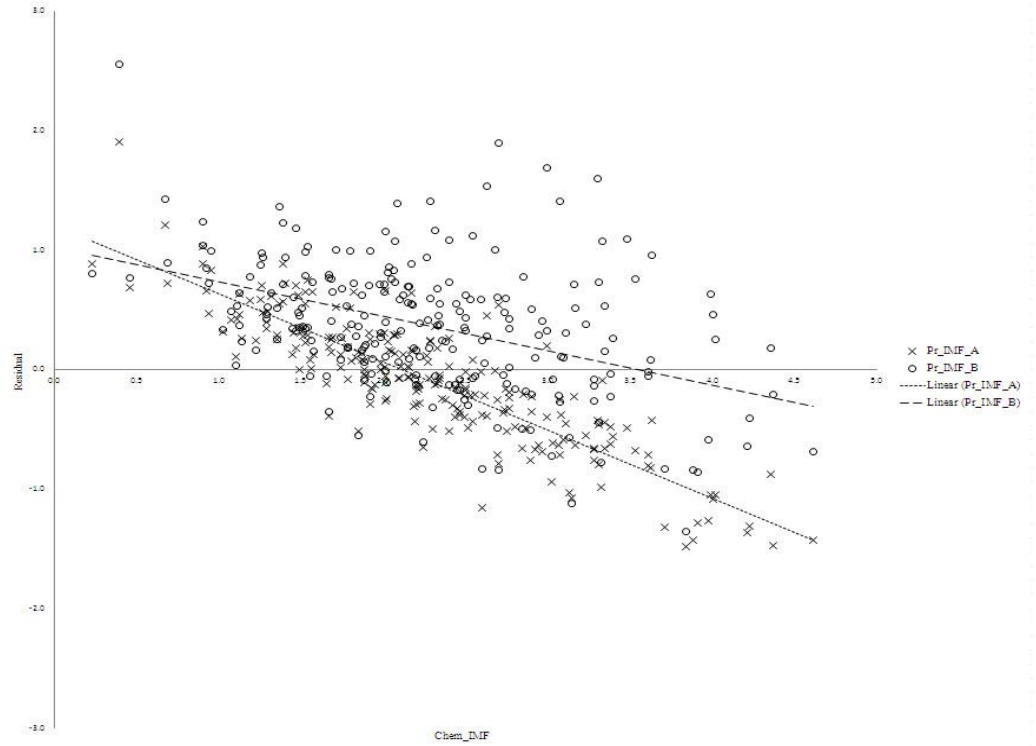


Figure 4.2 Fitted values of predicted IMF using both models (A and B) against Chem_IMF for the Tex development data (i), SBF data (ii) and the TexX data (iii)

(i)


Residual
Chem_IMF
Pr_IMF_A
Pr_IMF_B
Linear (Pr_IMF_A)
Linear (Pr_IMF_B)

(ii)


Residual
Chem_IMF
Pr_IMF_A
Pr_IMF_B
Linear (Pr_IMF_A)
Linear (Pr_IMF_B)

(iii)

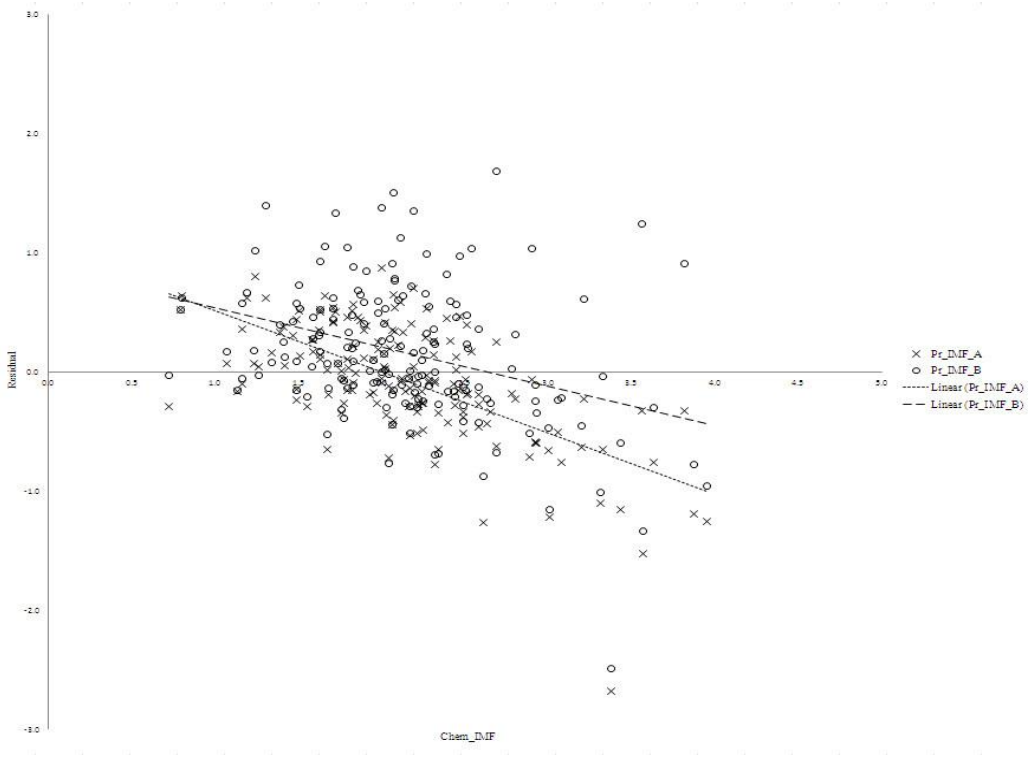


Figure 4.3 Residual values of predicted IMF using both models (A and B) against Chem_IMF for the Tex development data (i), SBF data (ii) and the TexX data (iii)

Table 4.5 compares the average absolute error of CT-predicted IMF in all three genotypes, as estimated by the two prediction equations, with Chem_IMF. Average absolute error is expressed as the mean of the error of prediction (residuals) expressed as IMF percentage, of the fitted values over Chem_IMF.

Model A performs better in both the SBF and TexX, with an average absolute error of 0.42 and 0.37 percentage points in SBF and TexX respectively and an average absolute error of 0.30 in the Tex data. Model B performs slightly better in the Tex data with an absolute error of 0.29, however in both the SBF and TexX data, model B has a slightly higher absolute error when compared to model A within the same genotype (SBF = 0.53, TexX = 0.45).

The phenotypic correlations between Chem_IMF and CT variables are presented in Table 4.6. This shows weaker phenotypic relationships between CT variables and Chem_IMF in the TexX data, compared to the other genotypes. However, although the strength of relationship differs across genotypes, the ranking remains similar. The exceptions are the relationship between age at CT and Chem_IMF and age at slaughter and Chem_IMF, where in the TexX data this relationship is positive, rather than negative as in the Tex and SBF data, however the relationship is very weak across all genotypes.

Table 4.5: Average absolute error, as the absolute mean of the magnitude of the residuals expressed as IMF percentage of the Pr_IMF (%) in both models (A and B) from Chem_IMF (%) in all three genotypes

Genotype	**Tex**	**SBF**	**TexX**
Model	Average absolute error	Average absolute error	Average absolute error
A	0.30	0.42	0.37
B	0.29	0.53	0.45

Table 4.6: Correlation (r) between Chem_IMF and CT traits employed in the prediction models within each data set

	Texel (n=370)	SBF (n=230)	TexX (165)
CTLWT	0.41	0.32	0.26
CTAGE	-0.14	-0.22	0.14
LV5MD	-0.71	-0.60	-0.46
TV8MD	-0.72	-0.56	-0.50
LV5STD	-0.76	-0.74	-0.58
TV8STD	-0.76	-0.73	-0.57
ISCSTSD	0.68	0.59	0.47
LV5STSD	0.65	0.68	0.52
TV8STSD	0.65	0.64	0.50
Pr_Cfat	0.71	0.74	0.54
SL_AGE	-0.11	-0.14	0.17
Model A	0.81	0.80	0.61
Model B	0.82	0.76	0.60

4.5 Discussion

Acknowledging that the structure of the data available was not optimal for the analysis of the effect of genotype and sex on both Chem_IMF and Pr_Cfat given the inclusion of castrates in the TexX data set, it is still possible to draw conclusions that there is an effect of genotype on both Chem_IMF and Pr_Cfat. It has been previously reported that to ensure consumer acceptability, a minimum level of 3% chemical IMF should be achieved in grilled cuts of lamb (Savell and Cross, 1988). Although, it should be noted that an up to date and comprehensive study on the relationship between IMF in lamb and consumer taste panel results in the UK has yet to be carried out and previous papers have highlighted the difference in preference of lamb meat in different countries (Sanudo et al., 1998). All three genotypes within this study fell below that recommended level at the point at which they were slaughtered, and the purebred Texels were significantly lower in both Chem_IMF and Pr_Cfat when compared to SBF and TexX. During a separate study employing the same data

it was shown that meat from the SBF lambs had higher tenderness, stronger lamb flavour and higher overall liking than the Tex lamb meat (Navajas et al., 2008). In rank order, SBF lambs had the highest levels of Pr_Cfat and Chem_IMF, followed by the commercial cross TexX and finally Tex lambs. This follows previous research reporting that breeding strategies in terminal sire breeds have focussed on increasing lean meat production while maintaining or reducing overall carcass fatness and, given the positive genetic correlation between overall carcass fatness and IMF in sheep (e.g. (Lorentzen and Vangen, 2012), this selection has resulted in extreme low IMF levels in terminal breeds such as the Texel.

In all genotypes (within genotype), males showed lower levels of Chem_IMF to that of females when adjusted for CTLWT. This difference was also seen in both Tex and SBF but not seen in TexX lambs when adjusted for Pr_Cfat with castrates showing higher levels of IMF when compared to females at the same Pr_Cfat, although this difference was not statistically significant. This probably reflects the fact that the males were castrated in the crossbred lambs, whereas SBF and Tex males were entire.

Females, within genotypes, also showed significantly greater levels of Pr_Cfat than males, when compared at the same live weight. These findings agree with previous literature reporting in several different breeds that female sheep are on average fatter than both castrated and entire males, with the latter being on average the leanest (Bass et al., 1990; Butler-Hogg et al., 1984; Dransfield et al., 1990; Kirton et al., 1982).

In the prediction of IMF across the three genotypes using information from CT, the differing results in the transferability of the models from the Tex across the two other genotypes were investigated further. It had been expected that the prediction equations would transfer better to the TexX lambs than the SBF lambs, as the TexX would be genetically more similar to the purebred Tex. Firstly, any obvious difference in summary statistics across data sets, for either CT or slaughter traits, was considered, and it was apparent that the data sets for

different genotypes did not share the same structure when considering age at CT and age at slaughter. Compared to the Tex data set used to derive the prediction equations, the SBF data had a higher mean and wider range of age at CT (mean = 145 days, SD = 23.86 vs.133 days, SD = 21.01) and age at slaughter (mean = 163 days, SD = 27.16 vs. 150 days, SD = 23.3). TexX had a higher mean age at CT than the Tex, but with much lower variation in age (mean = 144, SD = 4.62 vs. 133 days, SD = 21.01), whilst age at slaughter was similar, but again with much lower variation in TexX (mean = 149, SD = 4.56 vs. 150 days, SD = 23.3). The Tex data set, from which the prediction equations were derived, consisted of lambs slaughtered at different end-points; fixed age (n = 134) or target live weight and condition score (n = 236). The TexX lambs were slaughtered at a defined time point (weaning), whereas the SBF lambs were slaughtered at finishing (according to target weight and body condition score). To investigate if there was any age effect related to the difference in prediction ability across the two data sets, age at slaughter was tested as a variable in the models, resulting in no change in the difference in prediction accuracy across the two data sets. The SBF data was also truncated to mirror the mean and range of age at slaughter in the TexX data. However the prediction accuracies remained at a similar level.

It was also considered that there may be increases in accuracies of prediction using a breed specific approach in the formulation of prediction equations. To investigate any improvement this may make in the prediction of IMF in the TexX data (where the greatest reduction in accuracies were observed), breed specific coefficients were calculated using the same CT variables as in the development data. However, this also resulted in no improvement in accuracies.

It would appear that there are genotype differences in the relationships between CT variables and IMF, as shown in Table 4.6. CT variables were, in general, less correlated with IMF in SBF than Tex and correlations in TexX were lowest. However, the correlations ranked similarly across genotypes, suggesting that the same CT variables had the greatest predictive

ability across genotypes. These differences in relationships between Chem_IMF and CT variables across genotypes would explain the reduction in prediction accuracy when applying equations with the same CT variables.

A subsequent approach in future development of CT prediction equations for IMF may involve the inclusion of multiple genotypes within the data set with the aim of producing a multi-breed prediction equation, applicable across breeds.

The results of this study in the prediction of IMF using CT are comparative to that of previous studies in varying genotypes. Prediction accuracies here range from $R^2 = 0.33$ to 0.57. Accuracies of 33% were reported by Karamichou *et al* (2006) in Scottish Blackface lambs, using muscle density information from three reference scans. Lambe *et al* (2010a) reported achievable accuracies of 36% to 48% using varying amounts of fat and muscle density information from one single scan in purebred Texel lambs. Also from one single cross sectional scan, Macfarlane *et al* (2005) reported an accuracy of 57% in a study including three terminal sire breeds (Suffolk, Texel and Charollais). When considering these previously-achieved accuracies in varying breeds, and also considering that there are very few, if any, *in vivo* predictors of meat quality traits, prediction accuracies achieved in the divergent genotypes in this study (SBF and TexX) provide promising evidence that the current prediction equations estimate IMF with acceptable levels of accuracy across breeds.

4.6 Conclusion

Purebred Texels on average have lower levels of Chem_IMF and Pr_Cfat than TexX and SBF lambs. However, it should be noted that, within this study, all three breeds were below the recommended minimum levels of IMF found in the literature.

Within genotype, Tex and SBF females achieve higher levels of IMF than entire males at the same carcass fat while, in the TexX, castrated males appear to achieve similar levels of

Chem_IMF to that of females. In addition the results of this study show that prediction equations derived from a terminal sire (Tex) data set are transferable in the prediction of IMF in divergent breeds (SBF and TexX). The transferability and resulting accuracy of prediction was better in SBF than in TexX, however levels of accuracy were still at an acceptable level in the TexX. Most current terminal sire breeding programmes are focussed almost entirely on carcass traits and ignore traits concerned with eating quality such as IMF, largely due to the fact that there were few, if any, *in vivo* predictors of meat quality in sheep. These results provide evidence that prediction equations derived from purebred Texel lambs for successfully predicting IMF from CT variables can be transferred across divergent genotypes, providing potential for collection of meat quality data that could be incorporated into a wide range of breeding programmes.

Chapter 5: Preliminary genetic parameters of CT estimated traits and meat quality traits in Texel sheep

5.1 Summary

The data used in the previous chapters (Chapter 2 and 3) were used to produce genetic parameters for the novel IMF CT trait (PIMF1 and PIMF2) and to estimate genetic relationships between laboratory based methods of measuring meat (eating) quality (chemical IMF, shear force) which would be unavailable in any larger industry based data set, alongside growth and CT carcass composition. To enable this, the dataset was extended slightly to include animals scanned on the SRUC mobile scanner. Data were used from pure-bred Texel lambs (n=442) of both sexes (females and entire males), lambs were CT scanned at a mean age of 132 days (SD=19.5), Live weights at CT scanning and pre-slaughter were recorded and carcass fat weight as estimated by CT was calculated. Post-mortem laboratory measures of IMF and shear force were recorded in the loin, mean age at slaughter was 149 days (SD=21.6). As two different CT scanners were used, scanner specific prediction equations were developed for predicted IMF. The equations used for the data from the SRUC mobile CT scanner were;

*PIMF1= 5.834 + (Pr_Cfat*0.3268) - (LV5MD*0.0321) - (TV8MD*0.0915)*

*PIMF2 = 3.26 - (LV5MD*0.0561) - (TV8MD*0.0983) + (ISCMSD*0.1758) - (ISCFD*0.0437) - (LV5FD*0.0137) - (ISCFSD*0.0370) - (LV5FSD*0.0041).*

The equations used for the data from the SRUC fixed site scanner were;

*PIMF1 = 6.920 + (Pr_Cfat*0.2425) - (LV5MD*0.0654) - (TV8MD*0.0637)*

*PIMF2 = 7.26 - (LV5MD*0.0720) - (TV8MD*0.0611) + (ISCMSD*0.0748) - (ISCFD*0.02090) - (LV5FD*0.00758) - (ISCFSD*0.0344) - (LV5FSD*0.0324)*

Genetic analyses were first attempted using an animal model and subsequently using a sire model, fixed effects and covariates were analysed using ASReml 3.0 software. The pedigree of the animals (8 generations) consisted of a total of 3868 records, 156 sires and 1239 dams, lambs were the progeny of 17 sires and 296 dams.

Genetic analyses were largely unsuccessful with both univariate models (animal and sire) producing seemingly unreasonable heritabilities or non-convergence of models. As a result bivariate analyses were very difficult also and again largely unsuccessful. The main aim of the chapter was to make good use of the post-mortem meat (eating) quality measurements that were available. Given the restrictions of the data, in particular the combination of small animal numbers and that, in recent research it was discovered that the design and research objective of one study which contributed a number of animals to the dataset resulted in a pedigree structure that limits the effectiveness of this dataset for genetic parameter estimation. The results of this chapter are not sufficient to satisfy the main aims.

5.2 Introduction

In the previous chapters, it was shown that computed tomography information can provide very accurate *in vivo* estimates of IMF in the loin of Texel sheep. The aim of this chapter was to use the same data set to estimate heritabilities and genetic correlations between the available traits (i.e. chemical IMF, CT estimated IMF, mechanical shear force and growth and carcass composition traits). The primary objectives of this chapter were to estimate preliminary heritabilities of the novel CT-based predictions of IMF and laboratory based MQ measurements (chemical IMF and shear force) and estimate genetic correlations between these CT-based predictors and post-mortem laboratory based MQ measurements (chemical IMF and shear force), which would be unavailable in any larger industry based dataset.

5.3 Materials and Methods

Definitions of trait groups and variables used within the study are shown in Table 5.1.

5.3.1 Experimental animals

A full description of the data and detailed animal procedures can be found in Chapter 2, section 2.3.1. This study used the Texel data from both experimental farms described in Chapter 2, which includes data from the SRUC research farm in Scotland and the IBERS research farm in Wales.

The data comprised of pure-bred Texel lambs (n=442) of both sexes (females and entire males), reared to weaning as singles (n=239), twins (n=176) or artificially hand-reared (n=27). The mean age at CT was 132 days (SD=19.5, ranging from 93 to 202 days), and the mean age at slaughter was 149 days (SD=21.6, ranging from 99 to 234 days). In total there were records from 442 lambs, offspring of 17 sires, and 296 dams (Table 5.2).

5.3.2 Live animal and slaughter measurements

Live weights at CT scanning and at slaughter were recorded, alongside chemical IMF levels and mechanical shear force. Full details of methods used in the chemical extraction of IMF and mechanical measurement of shear force are described in Chapter 2. Total carcass fat weight was estimated by CT as described in Chapter 2.

Two different CT scanners have been used. Lambs reared within the SRUC experimental flock were scanned at the SRUC-BioSS CT unit in Edinburgh using a Siemens Somatom Esprit single slice CT scanner (n=370), whilst lambs reared at the IBERS experimental farm in Wales were scanned using a mobile GE LightSpeed 16 slice CT scanner (n=72). Full details of the animal procedures at scanning can be found in Chapter 2, section 2.3.2 and full details of slaughter and meat quality measurements can be found in Chapter 2, section 2.3.3.

Table 5.1: Group of traits and definition of variables included in the study

Trait group	Trait	Definition
Live weight	CTLWT	Live weight recorded at CT scanning
	SLWT	Live weight recorded pre-slaughter
Meat Quality	ShF	Mechanical shear force in the loin (kgF)
	IMF	Chemical intramuscular fat in the loin (%)
Computed Tomography	CTFW	Carcass fat weight estimated by CT (kg)
	PIMF1	CT predicted IMF (%) using equation 1, which included CTFW
	PIMF2	CT predicted IMF (%) using equation 2, which did not include CTFW

Table 5.2: Number of lambs for which CT was available alongside number of sires and dams within each year

Year	Lambs	Sires	Dams
2003	121	10	86
2004	115	10	80
2009	206	7	176
Total	442	17*	296*

*Sire and dam counts are not cumulative as sires and dams will have been used across years

5.3.3 CT estimates of intramuscular fat

As different scanners were used between farms, and as we know that there is a scanner effect on density values within soft tissue ranges (Bunger et al., 2010), scanner-specific equations were developed for the scanners used (A: fixed, or B: mobile).

5.3.4 Fixed scanner

Intramuscular fat levels in the loin were estimated from CT data using two separate prediction equations from Chapter 2 (section 2.5.1.4 and section 2.5.2.3 respectively). Firstly an equation including a CT estimate of total carcass fat (see Chapter 2, section 2.3.2), and

secondly using an equation independent of any CT fat weight or area measurements (PIMF1 and PIMF2 respectively).

The equations used were;

Scanner A: Siemens Somatom Esprit single slice CT scanner:

PIMF1 = 6.920 + (Pr_Cfat*0.2425) - (LV5MD*0.0654) - (TV8MD*0.0637)

PIMF2 = 7.26 - (LV5MD*0.0720) - (TV8MD*0.0611) + (ISCMSD*0.0748) - (ISCFD*0.02090) - (LV5FD*0.00758) - (ISCFSD*0.0344) - (LV5FSD*0.0324)

5.3.5 Mobile scanner

The mobile scanner equations were developed from the ones established for the fixed scanner. These were modified for the mobile scanner by fitting the optimal variables identified in Chapter 2 using a multiple linear model to an independent data set. The dataset consisted of 63 Texel lambs from the research farm in Wales (IBERS) and 72 purebred Texel lambs from the SRUC research farm in Scotland, all of which were CT scanned in 2009 using a mobile GE LightSpeed 16 slice CT scanner. The total data set included both females and entire males (n= 71 and 64 respectively), live weight at scanning ranged from 16.8kg to 47.6kg (SD = 5.65), chemical IMF ranged from 0.37% to 2.87% (SD = 0.62), Summary statistics of the CT traits and meat quality traits used in the determination of scanner specific prediction equations can be found in Table 5.3.

The terms included in the models were the same terms derived in the single ‘best’ model inclusive of CT predicted carcass fat and the single ‘best’ model independent of CT predicted carcass fat discovered in Chapter 2.

These were: Pr_Cfat, LV5MD, TV8MD, ISCMSD, ISCFD, LV5FD, ISCFSD and LV5FSD.

The terms from Chapter 2 described above were fitted in a multiple linear regression model in Genstat14^{TM} (Payne et al., 2012) in order to produce scanner specific coefficients and assess accuracy (R^2) within the same models. The prediction equations derived in this way

for the mobile CT scanner achieved R^2 (RMSEP) = 0.71(0.33) and 0.70 (0.34) for PIMF1 and PIMF2 respectively.

Scanner B: Mobile GE LightSpeed 16 slice CT scanner:

PIMF1= 5.834 + (Pr_Cfat*0.3268) - (LV5MD*0.0321) - (TV8MD*0.0915)

PIMF2 = 3.26 - (LV5MD*0.0561) - (TV8MD*0.0983) + (ISCMSD*0.1758) - (ISCFD*0.0437) - (LV5FD*0.0137) - (ISCFSD*0.0370) - (LV5FSD*0.0041)

Table 5.3: Trait descriptions, means and standard deviations of the dataset used in the development of mobile scanner prediction equations (SD) (n=135)

Trait	Acronym	Trait Description	Mean	SD
CT Traits				
	ISCMSD	Standard deviation of muscle density at ischium scan site (HU)	16.17	1.01
	ISCFD	Average fat density at ischium scan site (HU)	-47.36	6.11
	ISCFSD	Standard deviation of fat density at ischium scan site (HU)	28.21	1.95
	LV5MD	Average muscle density at 5th lumbar vertebra scan site (HU)	43.37	2.05
	LV5FD	Average fat density at 5th lumbar vertebra scan site (HU)	-45.38	4.09
	LV5FSD	Standard deviation of fat density at 5th lumbar vertebra scan site (HU)	28.71	1.28
	TV8MD	Average muscle density at 8th thoracic vertebra scan site (HU)	39.53	2.09
	Pr_Cfat	Predicted total carcass fat weight (kg)	1.49	1.07
MQ Traits				
	Shear force	Mechanically measured shear force in *M. longissimus lumborum* (kgF)	3.10	1.38
	IMF_Loin	*M. longissimus lumborum* intra-muscular fat (%)	1.32	0.62

5.3.6 Pedigree

The pedigree of the animals included in this study (8 generations) consisted of a total of 3868 records, 156 sires and 1239 dams. As described in Table 5.2, lambs were the progeny of 17 sires and 296 dams over three years.

5.3.7 Descriptive statistics

The means, standard deviations (SD) and coefficient of variation (CV) for the live weight, meat quality and CT traits in the study, across all 442 lambs from SRUC and IBERS, are shown in Table 5.4.

Mean chemically extracted IMF percentage was 1.46% (SD 0.67), mean IMF percentage estimated by CT was 1.45% in both PIMF1 and PIMF2 (SD 0.55 and 0.56 respectively), with a minimum of 0.04% 0.19% and a maximum of 3.33% and 3.22% for PIMF1 and PIMF2 respectively.

Table 5.4: Descriptive statistics for computed tomography traits

Trait group	Trait	n	Mean	SD	Minimum	Maximum	CV
Live weight	CTLWT	442	34.27	5.59	16.8	49.0	16.3
	SLWT	442	34.35	5.50	19.71	52.2	16.0
Meat Quality	ShF	442	3.43	1.57	1.39	10.72	45.84
	IMF	439	1.46	0.67	0.27	3.88	45.99
Computed Tomography							
	CTFW	431	2.25	1.12	0.03	6.86	50.03
	IMF1	442	1.45	0.55	0.04	3.33	38.24
	IMF2	442	1.45	0.56	0.19	3.22	38.48

Definitions of trait abbreviations can be found in Table 5.1.

5.3.8 Genetic analysis - Animal Model

Genetic analyses were first attempted using an animal model. Fixed effects and covariates were analysed using ASReml 3.0 software (Gilmour et al., 2009). The model fitted to live weights at CT and slaughter included fixed effects of year born (3 levels: 2003, 2004 or 2009), age of dam at lambing (6 levels: from 2 to 7 years), sex (2 levels: entire male or female), farm (2 levels: SRUC or IBERS), rearing rank (3 levels: single, twin or artificially hand reared) and a linear covariate of age at CT scanning or age at slaughter. Analysis of meat quality traits included the same fixed effects as the previous model, but a linear covariate of live weight at slaughter, rather than age, as they were considered to be slaughter traits (Kvame and Vangen, 2007). Significance of fixed effects and linear covariates fitted for each trait are shown in Table 5.5.

A mixed animal model was fitted including all fixed effects and linear covariates as described above.

$$Y = Xb + Za + e$$

Y is the vector of observations on the trait of interest, b is a vector of the fixed effects with associated matrix X. a is the vector of additive random animal (genetic) effects with associated matrix Z, and e is the vector of random residual effect.

Only significant fixed effects and linear covariates were fitted in the final models.

Table 5.5: Significance of fixed effects and covariates for each trait analysed

Trait group	Trait	Yrborn	DAMage	Sex	Farm	Rearing Rank	Cov^1	Cov^2	Cov^3
Live weight	CTLWT	***	ns	***	**	***		***	
	SLWT	***	ns	***	*	***	***		
Meat Quality	ShF	***	ns	***	***	***			***
	IMF	ns	ns	***	ns	ns			***
Computed Tomography									
	CTFW	***	ns	***	ns	***		***	
	PIMF1	***	ns	***	ns	***		***	
	PIMF2	***	ns	***	ns	***		***	

Cov^1 = Age at slaughter, Cov^2 = Age at CT scanning, Cov^3 = Live weight at slaughter, *= p<0.05, ** = p<0.01, *** = p<0.001

5.3.9 Genetic analysis - Sire Model

Following difficulties with the animal models (section 5.4.1), genetic analyses were then performed using a sire model. As was previously carried out and explained for the animal model in section 5.4.2, fixed effects and covariates were analysed using ASReml 3.0 software (Gilmour et al., 2009). Significance of fixed effects and linear covariates fitted for each trait are shown in Table 5.6.

A mixed sire model with pedigree was fitted including all fixed effects and linear covariates as they were described above.

$$Y = Xb + Zs + e$$

Y is the vector of observations on the trait of interest, *b* is the vector of fixed effects with associated matrix *X*. *s* is the vector of additive random sire (genetic) effects with associated matrix *Z*, and *e* is the vector of random residual effect.

Only significant fixed effects and linear covariates were fitted in the final models

Table 5.6: Significance of fixed effects and covariates for each trait analysed

Trait group	Trait	Yrborn	DAMage	Sex	Farm	Rearing Rank	Cov^1	Cov^2	Cov^3
Live weight	CTLWT	***	ns	***	***	***		***	
	SLWT	***	ns	***	*	***	***		
Meat Quality	ShF	***	ns	***	***	***			***
	IMF	ns	*	***	ns	ns			***
Computed Tomography									
	CTFW	***	ns	***	ns	***		***	
	PIMF1	***	ns	***	ns	***		***	
	PIMF2	***	ns	***	ns	***		***	

Cov^1 = Age at slaughter, Cov^2 = Age at CT scanning, Cov^3 = Live weight at slaughter

*= p<0.05, ** = p<0.01, *** = p<0.001

5.4 Results

5.4.1 Animal model results

5.4.1.1 Heritability estimates

Estimates of additive genetic variance (V_A), residual variance (V_R), phenotypic variance (V_P) and heritability (h^2) estimates for the live weight traits, *post-mortem* meat quality traits and *in vivo* CT traits from the univariate analyses are shown in Table 5.7.

Both models for CTLWT and chemical IMF failed to converge and were unable to produce estimates of the variance components or heritabilities. Very high heritabilities were estimated for SLWT, CTFW, PIMF1 and PIMF2 (0.88 to 0.98). The estimated heritability of ShF was very low (0.07) and not significantly different from zero with a S.E of 0.09.

Table 5.7: Estimated heritability's (S.E.) for the live weight, meat quality and computed tomography traits

Trait	CTLWT*	SLWT	ShF	IMF*	CTFW	PIMF1	PIMF2
V_A	21.18	23.50	0.13	0.33	1.01	0.27	0.23
V_R	0.00005	0.45	1.78	0.000001	0.05	0.01	0.03
V_P	21.18	23.94	1.91	0.33	1.06	0.28	0.26
h^2	1.00 (0.00)	0.98 (0.18)	0.07 (0.09)	1.00 (0.00)	0.95 (0.19)	0.95 (0.17)	0.88 (0.17)

***Model not converged**

5.4.1.2 Bivariate Analyses

Bivariate analyses were investigated using an animal model between the traits included in Table 5.1, with very little success (results not presented), producing unreasonable genetic parameters or non-convergence in all models.

The primary aim in these analyses was to estimate the genetic correlations between chemically extracted IMF measured *post-mortem*, and the CT predicted traits (PIMF1 and 2, CTFW), however these analyses were unsuccessful due to lack of convergence initially in the univariate model for one of the main traits of interest (IMF), although some univariate models did converge, and S.E were small in the estimates. The results were considered not to be accurate, given the unrealistically high h^2 estimates.

5.4.2 Sire Model Results

5.4.2.1 Heritability estimates

Estimates of variance components and heritability estimates for the live weight traits, *post-mortem* meat quality traits and *in vivo* CT traits from the univariate analyses are shown in Table 5.8.

High heritabilities were estimated for live weight traits (CTLWT and SLWT) as well as high heritabilities for chemically extracted IMF and CT estimated traits (CTFW, PIMF1 and PIMF2) ranging from 0.65 to 0.91. These heritabilities were all accompanied by large S.E, ranging from 0.26 to 0.32.

The heritability estimate for ShF was not significantly different from zero ($h^2 = 0.07$, S.E = 0.10).

Table 5.8: Estimated heritability's (S.E.) for the live weight, meat quality and computed tomography traits

Trait	CTLWT	SLWT	ShF	IMF	CTFW	PIMF1	PIMF2
V_{sire}	4.10	3.69	0.035	0.048	0.21	0.059	0.053
V_A	16.40	14.75	0.14	0.19	0.82	0.24	0.21
V_R	15.04	17.20	1.87	0.25	0.76	0.20	0.20
V_P	19.14	20.89	1.91	0.30	0.96	0.26	0.25
h^2	0.86 (0.31)	0.71 (0.28)	0.07 (0.10)	0.65 (0.26)	0.85 (0.31)	0.91 (0.32)	0.85 (0.31)

5.4.2.2 Bivariate Analyses

Again, bivariate analyses were investigated, this time using a sire model (results not presented). And as previously reported when applying an animal model, the sire model bivariate analysis produced seemingly unreasonable genetic parameters and non-convergence of models.

5.5 Discussion

The aim of this chapter was to make use of the *post-mortem* meat quality measurements that were available in the data set and investigate the genetic relationship of these and the CT estimated traits which may be obtained from current CT analyses, alongside the novel IMF estimates obtained as a result of the work in Chapter 2.

Given the restrictions of the data, in particular the combination of small animal numbers (n= 442), and, in very recent research using the same data set, it was discovered that the design and research objective of the study used to produce this data, in particular for the lambs produced in 2009 (Macfarlane et al., 2014), resulted in a pedigree structure that limits the effectiveness of this data set for genetic parameter estimation, since both males and females were intensively selected in order to increase the genetic frequency of carriers of the genetic trait of interest (Donaldson, 2015). During this study, it was highlighted that closely related individuals were used as parents in the 2009 trial and that the common sires may be traced back to a single sire. It was also highlighted that, as a result of very different breeding strategies across the two research interests (2003/2004 and 2009), there was very low connectedness between the two separate trial groups (Donaldson, 2015).

The closely related individuals included in the 2009 data (traced back to a single common sire) and the low connectedness between the datasets taken from each separate trial made the final aim of this chapter very difficult to achieve. Univariate heritabilities for the traits of interest were estimated, however, these were either seemingly unreasonable, in the case of both the animal model and sire model, and/or accompanied by very large S.E. Heritability results would have been expected to be close to the magnitude reported in previous studies across different genotypes, such as those, for example, reported by Karamichou *et al* (2006) for CT live weight in Scottish Blackface ($h^2 = 0.41$), Karamichou *et al* (2006) and Jones *et al* (2004) reported $h^2 = 0.30$ and 0.38 for pre-slaughter weight in Scottish Blackface and

Texel respectively, these and similar studies also reported heritabilities for shearforce ranging from 0.27 to 0.39 (Karamichou et al., 2006; Mortimer et al., 2014), chemically extracted IMF heritabilities ranged from 0.32 to 0.48 (Karamichou et al., 2006; Lorentzen and Vangen, 2012; Mortimer et al., 2014) and for CT estimated fat weight heritabilities ranged from 0.18 to 0.60 (Jones et al., 2004; Karamichou et al., 2006; Kvame and Vangen, 2007).

The genetic relationship between chemically extracted IMF, CT estimated IMF and CT estimated fat weight was of particular interest in these data to better understand the genetic relationships between CT estimated traits and laboratory based methods. However, robust and accurate heritability estimates for CT estimated IMF, and genetic correlations between this and the traits already included in the breeding programme, are the main focus to enable the inclusion of novel CT traits into current breeding programmes. The results of this chapter are not sufficiently accurate to fulfil these requirements. Therefore, a larger industry data set was made available. This made it possible to pursue this aim and successfully estimate genetic parameters for these variables in the next chapter.

5.6 Conclusion

The ultimate aim of this chapter was to determine the genetic relationships between *post-mortem* meat quality measurements that were available in the data set, the CT estimated traits obtained from current CT analyses and the novel CT estimated IMF calculated as a result of the work included in Chapter 2.

The structure of the dataset and the genetic relationships between animals included in the data, as outlined in the discussion, made the ultimate aim of the chapter with regards to the genetic relationships between traits unattainable. These relationships remain of particular interest to us and although this was not possible to estimate in the current study, further

studies may provide data sets with the required objective meat quality measures included in this study (and possibly more e.g. NIR) and CT data. Given a large enough data set with sufficient genetic variation and diversity of pedigree, the genetic relationships between these traits may be estimated.

Chapter 6: Genetic parameters for growth, carcass composition and intramuscular fat in Texel sheep measured by x-ray computed tomography and ultrasound

6.1 Summary

In previous chapters it has been reported that CT scanning provides very good in vivo estimates of IMF in the loin of Texel sheep. The next stage would be the inclusion of these estimates (IMF) into current commercial multi-trait selection indices. To enable this, genetic parameters including heritabilities and genetic relationships with other important traits included in the current indices are required. Industry based data were available for 1971 entire male lambs with CT scanning records from 2002 to 2013, the progeny of 525 sires and 1576 dams. Growth, CT and US measurements were available across the dataset. Growth measurements comprised of live weight at approximately eight weeks of age and twenty one weeks of age. Ultrasound data included muscle and fat depths at the third lumbar vertebra. And CT measurements included fat and lean weights, gigot muscularity, eye muscle area and the novel IMF estimates developed in Chapter 2. Mean intramuscular fat content as predicted by CT in PIMF1 and PIMF2 was 2.32% and 1.84% respectively (SD= 0.64 and 0.46), ranging from 0.62% to 5.12% and 0.26% and 3.60% respectively. Fixed effects and covariates were chosen based on the univariate analyses, variance and covariance components were then estimated applying restricted maximum likelihood (REML) procedures to fit a mixed linear animal model. Heritabilities were produced from the univariate analyses and phenotypic and genetic correlations between all growth, US and CT traits were calculated using the phenotypic and genetic variances and the phenotypic and genetic covariances from the bivariate analyses. Moderate heritabilities were estimated for growth traits, with moderate to high heritabilities estimated for US and CT traits. Heritability estimates for PIMF1 and PIMF2 were moderate and similar (h^2 = 0.36 and 0.31 respectively). Strong genetic correlations were seen between PIMF1 and CT and US fat traits (r_g = 0.83 and 0.64), whereas the same relationship was not seen in PIMF2 and CT and US fat traits (r_g=0.59 and 0.60). PIMF1 generally had a stronger genetic relationship

with growth, US and CT traits than PIMF2, however both traits were highly genetically correlated themselves (r_g=0.89).

This study is among the first to present genetic parameters of novel CT derived IMF estimates, which may be used as a proxy trait for meat eating quality and shows that accurate estimations of IMF are heritable and have the potential to be included into current selection methods. The parameters reported in this study can now be used to develop future breeding programmes.

6.2 Introduction

Chapters 2 and 3 have shown that CT scanning provides very good *in vivo* estimates of IMF in the loin of Texel sheep. These IMF estimates are of interest as an objective proxy trait in the determination of eating quality. Previously, accurate assessment of IMF in sheep was only possible with the slaughter, dissection and subsequent chemical analysis of the muscle of interest. The time and cost required complicate the inclusion of such a trait into breeding programmes. This non-destructive, *in vivo* method of estimation overcomes previous complications associated with destructive methods of measurement.

To enable the inclusion of novel CT traits, such as the IMF estimations developed in chapter 2 and 3, into selection indices, genetic parameters, such as heritabilities of the traits of interest and genetic relationships with traits included in the current indices, are required.

Currently a two-stage selection method is employed for US and CT scanning, during which all lambs are scanned and screened using US scanning prior to CT scanning as a result of the high cost and limited availability associated with CT scanning. If novel CT traits are to be included into current two-stage selection breeding programmes, genetic parameters for the novel CT traits and their correlations with traits already used in the genetic evaluation

(growth traits, US measurements and current CT estimations of carcass composition) are required.

Some of the previously reported genetic parameters including CT-measured tissue densities, carcass composition, meat quality traits *post-mortem* and organoleptic traits in Scottish Blackface lambs have been reported by Karamichou et al (2006), genetic parameters for US muscle and fat depths in Norwegian white sheep have been reported by Kvame and Vangen (2007), as well as a review of genetic parameter estimates which included US fat and muscle depths was carried out by Safari *et al* (2005) and a review of genetic parameters in meat quality traits was carried out by Hopkins (2011). And genetic parameters for meat quality traits post-mortem in Norwegian white X Texel lambs were reported by Lorentzen and Vangen (2012) as well as CT measures of muscularity in Suffolk, Charollais and Texel reported by Jones et al (2004). However the inclusion of novel traits into breeding programmes requires the estimation of genetic parameters specific to the breed and including those novel traits.

The aim of this chapter was to estimate the genetic parameters for CT-predicted IMF, including genetic correlations with current selection criteria (US tissue depths, CT carcass composition, muscularity and growth). Genetic parameters for the current selection criteria will also be confirmed. These parameters will enable future development of two-stage selection programmes in Texel sheep in order to improve or at least maintain meat eating quality (IMF) alongside the improvement of carcass composition traits such as lean meat yield, leanness and carcass quality.

6.3 Materials and Methods

Definitions of trait groups and variables used within the study are shown in Table 6.1.

6.3.1 Animals and BASCO Database

Data were extracted from the BASCO data Ltd. database, a national genetic evaluation database developed in 2004. Its purpose to store and manage very large amounts of pedigree and performance records in one single database. Originally including a co-operative of pedigree breeder associations, the Limousin cattle, and Texel and Suffolk sheep societies, pedigree and performance data is now stored on many more beef and sheep breeds.
The data set used here was restricted to Texel animals with CT scanning records and comprised records from 1971 entire male lambs from 525 sires and 1576 dams from 265 flocks over 12 years, of which 1957 animals also had records from ultrasound scanning and 1971 animals had records from CT scanning.

Full details of the number of lambs for which CT and US data were available, alongside the number of sires, dams and flocks within each year can be found in Table 6.2.

Table 6.1: Definition of variables included in the study

Trait group	Trait	Definition
Growth	8WWT	Live weight recorded at eight weeks of age
	21WWT	Live weight recorded at twenty one weeks of age (at the time of ultrasound scanning)
Ultrasound	USFD	Fat depth at the 3rd lumbar vertebra measured by ultrasound scanning (mm)
	USMD	Muscle depth at the 3rd lumbar vertebra measured by ultrasound scanning (mm)
Computed Tomography	CTFW	Carcass fat tissue weight estimated by CT (kg)
	CTMW	Carcass lean (muscle) tissue weight estimated by CT (kg)
	CTmusc	Muscularity score in the Gigot/Hind leg measured in the CT image taken at the ischium
	CTema	Area of *M. longissimus lumborum* (mm^2) measured in the CT image taken at the 5th lumbar vertebra
	PIMF1	CT predicted intramuscular fat percentage using equation 1 (%)
	PIMF2	CT predicted intramuscular fat percentage using equation 2 (%)

Table 6.2: Number of lambs for which CT and US data were available, alongside number of sires, dams and flocks within each year

Year	Lambs	Sires	Dams	Flocks
2002	87	34	76	26
2003	143	58	122	44
2004	204	70	178	61
2005	134	64	130	35
2006	89	40	82	23
2007	31	15	29	17
2008	88	33	85	21
2009	99	40	97	21
2010	166	55	155	40
2011	367	125	350	81
2012	318	97	280	71
2013	245	88	218	43
Total	1971	525*	1576*	265*

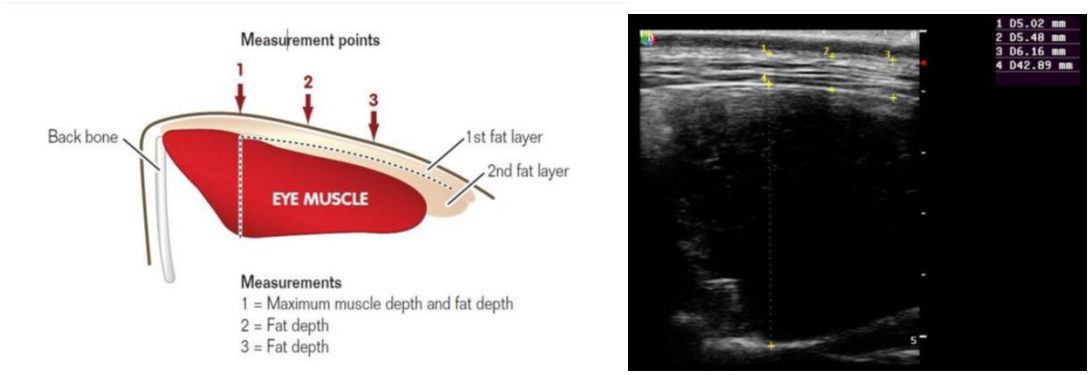
* Sires, dams and flocks are not cumulative as sires and dams will have been used across years, Flocks will record over several years

6.3.2 Growth measurements

Live weights are measured on farm at approximately eight weeks of age (mean = 66.4 days, range = 13 to 151 days) and included records from 1919 lambs, with a mean 8WWT of 31.28kg, ranging from 10.8kg to 68kg. As part of the commercial genetic evaluations in sheep, 8WWT is routinely adjusted for age (Moore, 2015) and 1867 records were available (mean = 27.26kg, range = 11.1 to 41.8kg). Live weight at twenty one week's was recorded either at twenty one weeks or at US scanning (mean = 143.3 days, range = 83 to 202 days), records were available for 1960 lambs, and mean 21WWT was 56.5kg, ranging from 26kg to 90kg.

6.3.3 Ultrasound measurements

Ultrasound data were available for lambs recorded between 2002 and 2013. Lambs were weighed and US scanned at an average age of approximately 150 days or 21 weeks. Muscle and fat depth (mm) were measured by US at the third lumbar vertebra. A single measure of muscle depth was taken, at the deepest point, and three measures of fat depth , with the first taken above the muscle at the deepest point and the following two measurements taken at 1cm lateral intervals from this point further from the backbone (Figure 6.1). All US scanning measurement, data capture and collation were carried out by Signet Breeding Services, part of EBLEX, the industry body for beef and lamb levy-payers in England.



a) **b)**

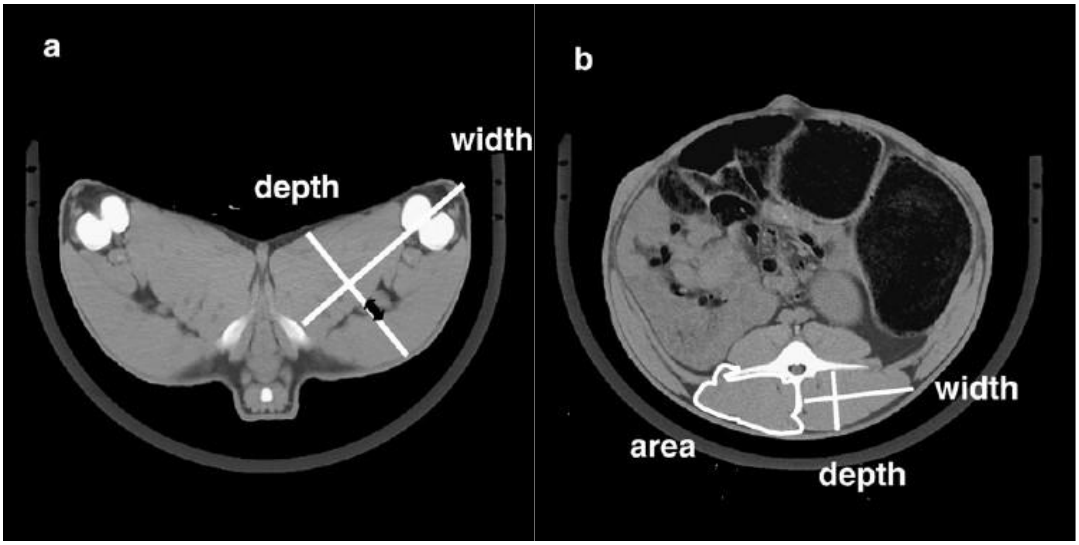
Figure 6.1 (a) Diagrammatical representation of measurement points taken at ultrasound scanning (b) Ultrasound scan image of measurement points taken at scanning (Images courtesy of Sam Boon, Signet)

6.3.4 Computed tomography measurements

From 2002 to 2013 lambs that were US scanned were then CT scanned at the SRUC-BioSS CT unit in Edinburgh using a Siemens Somatom Esprit single slice CT scanner or at various sites across the UK using a mobile GE LightSpeed 16 slice scanner. Full details of the animal procedures during scanning can be found in chapter 2.

All lambs were CT scanned within 2 weeks after US scanning. The CT image analysis for the measurement of fat and lean weights, percentages and ratios is described in detail in

chapter 2. Alongside these CT measurements, routine measurement of gigot muscularity and eye muscle area (cm^2) were taken as described in Jones *et al* (2002). In brief, the ratio of depth to width was taken from linear measurements on the scan image at the ischium, minus popliteal fat width, and multiplied by 100, then averaged over both legs providing a two-dimensional shape measurement in the gigot muscle (CTmusc, Fig. 6.2a). Area of the *M. Longissimus lumborum* (cm^2) on both sides of the image taken at the fifth lumbar vertebra was measured and averaged to give an eye muscle area measure (CTema, Fig 6.2b). A similar muscularity measurement based on the ratio of width to depth in the *M. Longissimus lumborum* was also taken and represented in Fig 6.2b, however was not used in this study.



a) **b)**

Figure 6.2 Measurements taken on the scan image taken at the ischium to calculate CTmusc (a) and measurements taken on the image taken at the fifth lumbar vertebra to calculate CTema (b) (Reproduced from (Lambe et al., 2007)

6.3.5 CT predictions of intramuscular fat

Intramuscular fat content in the loin was predicted using two separate prediction equations, firstly an equation including a CT prediction of total carcass fat weight (PIMF1) and, secondly, using a prediction equation entirely independent of any CT fat area or weight measurements (PIMF2). Both equations are described in chapter 2.

As different scanners were employed through the period of data collection from 2002-2013, and as we know that there is a scanner effect on density values within soft tissues (Bunger et al., 2010), scanner-specific equations were developed for scanner type used (A: fixed, or B: mobile, see Chapter 5, section 5.3.3.2).

To serve as a brief reminder, and also to detail the scanner specific equations, the equations used were:

For scanner A (Fixed scanner):

PIMF1 = 6.920 + (Pr_Cfat*0.2425) - (LV5MD*0.0654) - (TV8MD*0.0637)

PIMF2 = 7.26 - (LV5MD*0.0720) - (TV8MD*0.0611) + (ISCMSD*0.0748) - (ISCFD*0.02090) - (LV5FD*0.00758) - (ISCFSD*0.0344) - (LV5FSD*0.0324)

And for scanner B (Mobile scanner):

PIMF1= 5.834 + (Pr_Cfat*0.3268) - (LV5MD*0.0321) - (TV8MD*0.0915)

PIMF2 = 3.26 - (LV5MD*0.0561) - (TV8MD*0.0983) + (ISCMSD*0.1758) - (ISCFD*0.0437) - (LV5FD*0.0137) - (ISCFSD*0.0370) - (LV5FSD*0.0041)

Full details on how these equations were derived and the methods used to estimate intramuscular fat in the loin using available CT variables are presented in chapter 2.

6.3.6 Statistical analysis

Fixed effects and covariates affecting the traits of interest were identified using ASReml (Release 3.0) software (Gilmour et al., 2009). The model for growth traits (8WWT; 21WWT) included fixed effects of birth type (5 levels, 0-4) coding ‘unknown’ as 0 and single to quads as 1 to 4 (0 = 283 records, 1 = 685, 2 = 829, 3 = 167, 4 = 7), age of dam at lambing (9 levels; 1year old = 18 records; 2 years old = 541; 3 years old = 583; 4 years old = 414; 5 years old = 219; 6 years old = 118; 7 years old = 42; 8 years old = 26; 9 years old = 9 and 1 unknown, which was treated as a missing value), flock (265 levels), and year of birth (12 levels, 2002-2013). A flock by year interaction was fitted to account for any interaction between year and management and/or geographical climatic effects. A linear covariate of age

at measurement was fitted to 21WWT. No linear covariate was fitted to 8WWT as the data used was previously adjusted for age.

The model for US traits included the same fixed effects and the linear covariate fitted was age at ultrasound scanning. The model for CT-derived traits included fixed effects of birth type, age of dam at lambing, scanner type used (1 = Somatom Esprit single slice scanner, 2 = GE LightSpeed 16 slice scanner), flock, year of birth, flock by year interaction and a linear covariate of age at CT scanning.

Variance and covariance components were estimated from the data using ASReml (Release 3.0) software (Gilmour et al., 2009) applying restricted maximum likelihood (REML) procedures to fit a mixed linear model. The average information was used to maximise the likelihood. Using the variance component estimates provided from the univariate analysis an estimate of heritability (h^2) for each trait of interest was calculated as the ratio of the additive genetic variance (V_A) and the phenotypic variance ($V_P = V_A + V_R$), where:

$$h^2 = V_A/V_P$$

In addition to the univariate analysis, bivariate analyses were also performed between all combinations of growth, US and CT traits included in the study. The mixed animal model included all fixed effects and covariates as they were described above, as described by the equation:

$$Y = Xb + Za + e$$

Where Y is the vector of observations on the trait of interest, b is the vector of fixed effects with associated matrix X, a is the vector of additive random animal (genetic) effects with associated matrix Z, and e is the vector of random residual effect.

In order to maintain parity between models within trait groups, all fixed effects included in the univariate analyses were kept in the models for the bivariate analysis whether significant

or not, with exception of age of dam at lambing which was not included in the model for ultrasound traits (USMD and USFD) as it was not significant within that trait group.

Phenotypic (r_P) and genetic (r_G) correlations between all growth, US and CT traits were calculated using the phenotypic and genetic variances (V_P and V_A) and the phenotypic and genetic covariances (COV_P and COV_A) from the bivariate analysis. Phenotypic correlations (r_P), between traits were calculated as the ratio of the total phenotypic covariance (COV_P) between traits and the square root of the product of the total phenotypic variance (V_P) of both traits.

$$r_P = \frac{COV_{P\ (trait\ 1,2)}}{\sqrt{V_{P\ (trait\ 1)} * V_{P(trait\ 2)}}}$$

Genetic correlations (r_G) between traits were calculated as the ratio of the additive genetic animal covariance (COV_A) between traits and the square root of the additive genetic animal variance (V_A) of both traits.

$$r_P = \frac{COV_{A\ (trait\ 1,2)}}{\sqrt{V_{A\ (trait\ 1)} * V_{A(trait\ 2)}}}$$

6.4 Results

6.4.1 Descriptive statistics

Significance of fixed effects and linear covariates fitted in the univariate analysis are presented in Table 6.3.

The means, standard deviations (SD) and coefficient of variation (CV) for the growth, US and CT traits used in the study are shown in Table 6.4. Mean intramuscular fat percentage as predicted by CT in PIMF1 and PIMF2 was 2.32% (SD 0.64) and 1.84% (SD 0.46) respectively, with a minimum of 0.62% and 0.26% respectively and a maximum of 5.12% and 3.60% respectively.

Table 6.3: Significance from the univariate analyses of fixed effects and covariates for each growth, US and CT trait analysed

Trait group	Trait	Birth Type	Dam Age	Scanner	Flock	Year	Flock x Year	Cov^1	Cov^2
Live weight	8WWT	***	***		***	*	***		
	21WWT	***	***		***	*	***	***	
Ultrasound	USMD	***	ns		***	***	***	***	
	USFD	***	ns		***	***	***	***	
Computed Tomography	CTFW	***	**	***	***	***	***		***
	CTMW	***	**	**	***	ns	***		***
	CTmusc	**	ns	***	***	ns	***		***
	CTema	***	ns	*	***	ns	***		***
	PIMF1	***	**	***	***	**	***		***
	PIMF2	***	**	ns	***	ns	***		***

Cov^1 = Age at US scanning, Cov^2 = Age at CT scanning

ns = non-significant, * = p<0.05, ** = p<0.01, *** = p<0.001

6.4.2 Heritability estimates

Estimates of variance components and heritability estimates for the growth and *in vivo* US and CT traits measured are shown in Table 6.5. Moderate heritabilities were estimated for growth traits tested in the study, with moderate to high heritabilities estimated for US and CT traits. Heritability estimates for the novel CT predicted IMF traits were moderate and similar: h^2 0.36 ± 0.07 for PIMF1 and h^2 0.31 ± 0.07 for PIMF2.

6.4.3 Correlation estimates

6.4.3.1 Genetic correlations

Estimates of genetic correlations amongst growth, US and CT traits, including the novel intramuscular fat estimations from CT, are shown in Table 6.5. Correlations from 0.1 to 0.3 were considered weak, from 0.4 to 0.6 moderate and correlations greater than 0.6 were considered as strong, correlations with a S.E. greater than the correlation coefficient were not significantly different from zero.

Strong positive genetic correlations were found between 8WWT and 21WWT (r_g 0.64 ± 0.11), and between 8WWT and CTFW (r_g 0.77 ±0.12). Genetic relationships between 21WWT and CTMW were also strong and positive (r_g 0.76 ± 0.06). Genetic relationships between US and CT carcass fat measurements (USFD, CTFW) were strong and positive (r_g 0.61 ± 0.09), and strong positive genetic correlations were estimated between USFD and PIMF1 and PIMF2 (r_g 0.64 and 0.60).

Genetic correlations between USMD and CTema were strong and positive (r_g 0.78 ± 0.08), while the relationship between USMD and CTMW was positive and moderate (r_g 0.59 ± 0.11).

A strong positive genetic correlation was found between CTFW and PIMF1 (r_g 0.83 ± 0.04), and a moderate positive correlation was found between CTFW and PIMF2 (r_g 0.59 ± 0.10).

The genetic correlations between PIMF1 and the remaining current index traits (8WWT, USMD, USFD, CTMW, CTmusc) are low to moderate ranging from r_g 0.19 to 0.64 and stronger than the correlations seen between the same index traits and PIMF2 which were only significant in 8WWT (r_g 0.24 ± 0.18) and USFD (r_g 0.60 ± 0.11) (Table 6.5), with the muscularity traits (CTMW and CTmusc) not significantly correlated with PIMF2. The genetic correlation between PIMF1 and PIMF2 was strong and positive (r_g 0.89 ± 0.03).

6.4.3.2 Phenotypic correlations

Phenotypic correlation estimates among the growth, US and CT traits were consistent with the direction and magnitude of the corresponding genetic correlations (Table 6.5). Strong phenotypic correlations were found for pairings of traits with strong genetic correlations and generally the phenotypic correlations were smaller than the corresponding genetic correlation estimates.

Table 6.4: Descriptive statistics for growth, ultrasound and computed tomography traits

Trait group	Trait	n	Mean	SD	Minimum	Maximum	CV (%)
Live weight	8WWT	1867	27.26	4.34	11.1	41.8	15.9
	21WWT	1959	56.46	8.39	26	90	14.9
Ultrasound	USFD	1957	3.01	1.39	0.4	9.5	46.4
	USMD	1957	32.7	3.38	20.3	43	10.4
Computed Tomography	CTFW	1971	5.19	1.67	1.26	11.57	32.2
	CTMW	1971	17.52	2.54	9.36	25.32	14.5
	CTmusc	1971	67.93	6.94	40	86	10.2
	CTema	1971	27.45	4.29	14.35	44.4	15.6
	PIMF1	1971	2.32	0.64	0.62	5.12	27.7
	PIMF2	1971	1.84	0.46	0.26	3.60	25.2

Definitions of trait abbreviations can be found in Table 6.1

Table 6.5: Variances, phenotypic correlations and genetic parameters (S.E) for the growth, ultrasound and computed tomography traits

Trait	8WWT	21WWT	USFD	USMD	CTFW	CTMW	CTmusc	CTema	PIMF1	PIMF2
V_A	2.96	10.53	0.47	2.50	0.48	0.93	14.18	4.31	0.08	0.05
V_P	11.72	28.92	1.17	7.85	1.45	2.60	33.48	11.76	0.22	0.16
8WWT	**0.25 (0.07)**	0.57 (0.02)	0.13 (0.03)	0.19 (0.03)	0.48 (0.02)	0.51 (0.02)	0.13 (0.03)	0.29 (0.03)	0.40 (0.02)	0.26 (0.03)
21WWT	0.64 (0.11)	**0.36 (0.06)**	0.45 (0.02)	0.43 (0.02)	0.73 (0.01)	0.81 (0.01)	0.27 (0.03)	0.51 (0.02)	0.57 (0.02)	0.36 (0.02)
USFD	ns	0.42 (0.11)	**0.40 (0.07)**	ns	0.65 (0.02)	0.32 (0.03)	0.12 (0.03)	0.25 (0.03)	0.57 (0.02)	0.51 (0.02)
USMD	0.21 (0.18)	0.52 (0.12)	ns	**0.32 (0.07)**	0.37 (0.02)	0.50 (0.02)	0.27 (0.03)	0.66 (0.02)	0.21 (0.03)	0.12 (0.03)
CTFW	0.77 (0.12)	0.66 (0.08)	0.61 (0.09)	0.48 (0.13)	**0.33 (0.07)**	0.63 (0.02)	0.24 (0.03)	0.44 (0.02)	0.88 (0.01)	0.71 (0.01)
CTMW	0.43 (0.13)	0.76 (0.06)	0.15 (0.13)	0.59 (0.11)	0.49 (0.10)	**0.36 (0.06)**	0.33 (0.02)	0.69 (0.01)	0.43 (0.02)	0.21 (0.03)
CTmusc	0.20 (0.16)	0.44 (0.12)	ns	0.39 (0.12)	0.41 (0.13)	0.51 (0.11)	**0.42 (0.07)**	0.36 (0.02)	0.15 (0.03)	0.09 (0.03)
CTema	0.34 (0.16)	0.54 (0.10)	0.25 (0.14)	0.78 (0.08)	0.50 (0.12)	0.71 (0.07)	0.48 (0.11)	**0.37 (0.06)**	0.24 (0.03)	0.11 (0.03)
PIMF1	0.49 (0.15)	0.44 (0.11)	0.64 (0.10)	0.24 (0.15)	0.83 (0.04)	0.20 (0.13)	0.19 (0.14)	ns	**0.36 (0.07)**	0.90 (0.01)
PIMF2	0.24 (0.18)	ns	0.60 (0.11)	ns	0.59 (0.10)	ns	ns	ns	0.89 (0.03)	**0.31 (0.07)**

Heritabilities are in bold on the diagonal, genetic correlations below the diagonal and phenotypic correlations are above. All abbreviations are defined in Table 6.1. Correlations with S.E. greater than the correlation coefficient were not significantly different from zero (ns)

6.5 Discussion

This study is among the first to present heritabilities, and genetic and phenotypic correlation estimates of novel CT derived estimates of intramuscular fat as a proxy trait for meat eating quality. And also to present genetic relationships with routine growth, US and CT measurements taken as part of sheep performance recording schemes run by various breed societies in the UK.

Until recently there has been very little published literature on genetic variation for meat quality traits in sheep (Safari et al., 2005). In recent years several studies have presented genetic parameters for meat quality, US and CT traits in various sheep breeds (Jones et al., 2004; Karamichou et al., 2006; Kvame and Vangen, 2007; Lorentzen and Vangen, 2012; Mortimer et al., 2010; Mortimer et al., 2014).

The traits of particular interest during this study were the novel CT predicted intramuscular fat levels. The heritabilities of these novel traits, and their relationships with traits currently included in the selection index for terminal sires, are of particular interest. These results allow us to better understand whether these novel traits are, firstly, heritable and therefore selection based on these CT traits will result in genetic gain, and secondly, whether the relationship between these traits and current CT traits would result in any adverse effects due to selection for one or the other.

The improvement of IMF levels in the Texel breed appears to be necessary, considering the mean level of predicted IMF in the study, using either prediction equation, was below the minimum recommended level (3%) reported by Savell and Cross (1988) for grilled cuts of lamb, and well below the recommended level for ‘good every day’ eating quality in lamb (5%) reported by Hopkins et al (2006). These IMF levels, in commercial Texel ram lambs, are consistent with mean IMF levels for Texels in the research projects reported in chapter 4.

6.5.1 Heritability estimates

The CT and ultrasound traits included in this study were found to be of moderate to high heritabilities, implying that they can be improved through selection. Heritability estimates for USFD and USMD were moderate (0.40 and 0.32 respectively). For USFD, these values were consistent with previously published estimates in several studies and breeds, ranging from h^2 0.15 to 0.54 and heritability estimates for USMD were at the upper end of the range of published estimates (h^2 0.23 to 0.40) (Jones et al., 2004; Kvame and Vangen, 2007; Mortimer et al., 2010; Simm et al., 2002). For many of the of the studies reported, the breeds used were different to the breed used in this study, other than in Jones et al (2004), in which age at measurement was also used in the model. In this context it is of note that Kvame and Vangen (2007) reported, the design of an experiment, the environment, the breed and the apparatus used (including the variation in operator) have an effect on the results. In the present study, the use of records from animals selected based on US results, as part of the two stage selection will have an effect, as these animals will artificially have a related effect on the 'population' mean of the traits included in the selection.

Kvame and Vangen (2007) also reported that including either live weight at weaning or age at weaning in two different models had an effect on the genetic parameters for the same traits. The selection of live weight or age as a covariate depends on the trait of interest. If the trait of interest is a slaughter trait, live weight may be a more suitable covariate, given that the breeding objective would be to achieve the desired gain in the trait of interest at a given live weight. Alternatively, if the trait of interest is a growth trait, the more suitable covariate seems to be age at measurement, as the breeding goal aims to target the trait at a certain period in the animal's life.

In this study, age was fitted as a covariate in growth (where applicable), US and CT traits, as the US traits in this study were age related (21 week recorded). The CT traits, in some cases,

also included CT live weight as part of the model to estimate the trait of interest (i.e. CTFW and CTMW include live weight at CT in the prediction equation; see chapter 2, section 2.3.2).

The heritability estimates for CT traits in this study were also moderate to high, and estimates produced during this study were consistent with reported heritabilities in previous studies.

Previously published heritability estimates for CT measured muscle and fat weights ranged from 0.21 to 0.57 and 0.29 to 0.60 respectively (Jones et al., 2004; Karamichou et al., 2006; Kvame and Vangen, 2007; Lambe et al., 2008a). The estimates found in this study for Texel sheep were very similar to estimates reported by Jones et al (2004) in the same breed however the CT muscle weight heritability estimate was slightly lower than those reported for Scottish Blackface sheep by Karamichou et al (2006) and a terminal sire line by Kvame and Vangen (2007),. Both of these studies used different breeds and also reported higher standard errors in the estimations.

The only published heritability estimates that could be found for the CT muscularity traits used in this study were from the studies in which they were developed, Jones et al (2004) reported h^2 of 0.33 and 0.21 for CT eye muscle area and gigot muscularity respectively.

Given the novelty of the trait, estimated heritabilities for CT predicted IMF can only be compared with published *post-mortem*, chemically extracted IMF heritabilities. The heritability estimates produced in this study were similar to those found in previous studies, Lorentzen and Vangen (2012) and Mortimer et al (2014) both reporting h^2 of 0.48, and Karamichou et al (2006) reporting h^2 of 0.32. The similarity between heritabilities for CT based IMF and chemical IMF is indicative of the measurement accuracy of CT predictions of IMF in Texel sheep. If these predictions were not accurate, we would expect lower heritabilities as the errors in the methods of measurement would inflate the environmental component of the phenotypic variance and as a result lower the heritabilities.

6.5.2 Genetic correlation estimates

From the current results, selection for USFD would be expected to yield moderate changes in PIMF1 and PIMF2 (r_g 0.64 ± 0.10 and 0.60 ± 0.11). Moderate changes when selecting for USFD would also be expected in CTFW (r_g 0.61 ± 0.09). Some similar relationships of the same direction and magnitude were found in Jones et al (2004) reporting a moderate genetic correlation between USFD and CTFW (r_g 0.58). Lambe et al (2008b) reported similar moderate genetic correlations in Scottish Blackface sheep, between USFD and CTFW (r_g 0.62) and also a weak negative genetic correlation between USFD and CTMW (r_g -0.16). This study found strong positive genetic correlations of USFD with PIMF1 and PIMF2 (r_g 0.64 and 0.60), indicating that any selection to reduce USFD would see a relative reduction in IMF levels in the loin of Texel sheep, unless both traits are included in the multi-trait selection index.

Selection for USMD would be expected to yield moderate changes in CTFW (r_g 0.48 ± 0.13) and CTMW (r_g 0.59 ± 0.11) and was highly genetically correlated with CTema (r_g 0.78 ± 0.08) and moderately correlated with CTmusc (r_g 0.39 ± 0.12), indicating that any selection for increased USMD will see a corresponding increase in CT muscularity traits. The relationships between USMD and both PIMF1 and PIMF2 were close to zero or not significant, as expected, so no change in IMF would be predicted from selection to increase USMD.

The consistent and moderate to strong relationships between USMD and CTMW, and USFD and CTFW are important in a two stage selection programme. These moderate to strong relationships also indicate that US scanning is a useful technology for selection of carcass muscle and fat in the absence of the availability of CT scanning. Of particular interest, is the genetic relationship between the CT predicted IMF traits and current fat traits measured by both US scanning and CT scanning. PIMF1 was also very highly genetically correlated to

CTFW (r_g 0.83 ± 0.04), whilst PIMF2 had a less strong relationship (r_g 0.59 ± 0.10) with CTFW. These genetic relationships reflect the inclusion of CT predicted total carcass fat weight in the model used to estimate PIMF1 but not PIMF2. The relationship between PIMF1 and CTFW (r_g = 0.83) is larger than the genetic correlations found between chemically-extracted IMF and carcass fat measures found in previous studies. Lorentzen and Vangen (2012), reported r_g 0.62 ± 0.34 in Norwegian white cross Texel sheep (note the high standard error). Whereas the relationship between PIMF2 and CTFW was similar to those found between chemically-extracted IMF and carcass fat measures. The genetic correlations between PIMF1 or PIMF2 and CTFW indicate that PIMF2 is more independent of total carcass fat (r_g 0.83 vs. r_g 0.59). The results of this study indicate that selection to increase IMF based on either of the CT models would result in an associated increase in CTFW, which would be greater as a result of selection on PIMF1 than PIMF2. PIMF1 had moderate positive genetic correlations with growth (8wwt and 21wwt, r_g 0.49 and 0.44, respectively) whereas PIMF2 seems to be lower or not correlated with the weight traits (r_g 0.24 and ns from zero).

Index selection provides the opportunity to select simultaneously for several traits using a multi-trait selection index, in which selection traits are weighted depending on the ultimate breeding goal, and the results of this study suggests the integration of PIMF2 would be more independent of the current selection criteria rather than PIMF1, and any selection against a positive correlation will diminish the selection response.

Any larger increase in carcass fat weight is of course antagonistic with current selection practices, which attempt to reduce fat trimming in abattoirs. However these relationships also highlight that continued selection for leaner carcasses, without consideration of the correlated changes in IMF will further reduce the already extremely low levels of IMF in Terminal Sire breeds, in this case the Texel sheep.

6.5.3 Incorporating CT predicted IMF into selection programmes

The results of this study suggest that CT predicted IMF should be immediately incorporated into current selection programmes for Terminal Sire breeds that include reducing total carcass fat weight amongst other selection objectives. The goal of including such a trait into current breeding objectives would be to maintain or marginally improve the levels of IMF in the muscle of Texel sheep. Given that there is a large amount of variation in both CTFW and PIMF in the population used in this study, and the genetic correlation is significantly different from unity, suggests the two fat depots are at least partially under different genetic control and are not pleiotropic. As a result, there may potentially be selection candidates within the population (currently selected for leanness), these 'correlation breakers' fit the criteria for leanness and optimal levels of IMF.

6.6 Conclusion

This study shows that accurate estimations of IMF produced from CT technology are heritable and have the potential to be included in current two-stage selection programmes for Texel sheep in the UK. The incorporation of CT-measured fat and muscle weights as selection criteria has already increased the genetic gain in carcass composition traits over the last few years and will continue to do so. The inclusion of a proxy meat (eating) quality trait, such as CT predicted IMF, will add further value to the benefits of CT scanning and ensure the halt of further decreases, and would facilitate the quantification and possible improvement of IMF levels in the loins of Texel sheep.

The integration of this novel trait would be relatively straight forward in a practical sense, and the clients of the current system are already opting to send elite rams for CT scanning. This novel and commercially important trait will only increase the opportunities presented to breeders as a result of CT scanning.

The parameters reported in this study can now be used to develop two-stage selection programmes for the Texel genotype and potentially others (see chapter 4). This will enable breeders to make the best use of CT scanning technology to improve carcass composition without compromising, or at the very least, maintaining eating quality.

Chapter 7: General Discussion

7.1 Introduction

Given the moderate to high relationships found between CT measures and some MQ traits in sheep (Karamichou et al., 2006; Lambe et al., 2010a; Macfarlane et al., 2005; Navajas et al., 2008; Young et al., 2001), the initial aims of this project were to investigate the best image analysis methods using two-dimensional and/or three-dimensional CT information. Firstly to optimise the accuracy of CT as a non-invasive, *in-vivo* method to predict aspects of meat quality traits (intramuscular fat and mechanical shear force) in Texel sheep and secondly, to use the most accurate and precise methods of predicting aspects of meat quality and apply these across different breed types for which data may be available. Finally, these selected and validated prediction methods were then used to estimate genetic parameters for these novel CT traits and current traits which are recently included in the selection of elite sires within the Texel breeding industry, to provide both a set of genetic parameters for already integrated breeding traits and describing the genetic basis of any new, novel CT traits developed as a result of this project.

Each of the previous chapters including any experimental work also includes a comprehensive discussion as part of the chapter. Therefore the purpose of this general discussion is to summarise the findings and highlight the key points from each of the experimental chapters, furthermore, to evaluate the contribution made by these chapters and assess any limitations in the research. These findings will be discussed in the context of the UK sheep industry and the emphasis will be on the use of CT scanning as a tool for selection for MQ traits in Texel sheep. The general discussion will then be followed by consideration of the benefits to the sheep breeding industry of this work and finally highlighting areas of potential research in the future.

The project aimed to address the hypotheses that:

1. Two and three-Dimensional x-ray computed tomography can provide an accurate method for estimating MQ traits in live Texel sheep.
2. Resulting MQ predictors can be incorporated into current sheep breeding programmes, allowing continued improvements in growth and carcass traits, whilst maintaining or improving aspects of MQ

7.2 CT as a method for estimating MQ traits in Texel sheep

Computed tomography has been used in terminal sire sheep breeding programmes for the last few decades, with elite rams from many terminal sire breeds (e.g. Texel, Suffolk and Charollais) now routinely scanned. Carcass fat and lean weight can be predicted with very high accuracy (98-99%) using CT (even just using the Reference scan method), and in order to increase the viability and value of CT scanning selection programmes, novel and economically important CT based phenotypes, should be included in current two-stage selection programmes. Such novel phenotypes include MQ traits such as shear force and IMF. The current factors affecting lamb carcass value are carcass weight, conformation and carcass fatness, thus systems aiming to produce high quality carcasses have currently focussed on these economically important traits. The consideration of MQ factors (e.g. shear force and IMF) has until now been limited to the measurement of such traits *post-mortem* which is both expensive and destructive, and in turn limits the inclusion of these traits into current selection programmes. It has been shown that CT provides an opportunity to overcome these previous limitations in some MQ traits.

7.2.1 Shear force

Throughout the study in both Chapters two and three, CT predictors did not explain much of the variance in shear force with a maximum Adj R^2 of 0.14 (RMSEP = 0.15) using

information from routine reference scan images and no significant improvement was seen when spiral CT scan images were used. This objective MQ trait is understandably easily related to organoleptic traits such as tenderness by the consumer, and would be easily marketable as a 'proxy' trait for eating quality in live lambs. However the lack of accuracy achieved in this project does not provide sufficient confidence in the ability of CT to predict shear force.

The inability of *in vivo* CT to predict the *post-mortem* trait shear force may be due to the chemical and compositional changes that occur during the processing, cooking and ageing of a sample of meat (in this case lamb loin), which are part of the experimental process during shear force studies and are also important prior to retail marketing of red meat and during cooking by the consumer. These chemical and compositional changes include cooking loss, ultimate pH, drip loss, and *post mortem* glycolysis. Factors that also have an effect on shear force can be muscle fibre type and size, and connective tissue content. All these factors contribute to the ultimate values of shear force and chemical and compositional changes mean that muscle *post-mortem* is far removed from the same skeletal muscle *in-vivo*. Mechanically measured shear force is also known to have low repeatability which may also partially explain the low predictability. CT was unable to predict such a 'tangible' trait *in-vivo*, however with further work it may be possible to develop a method and increase the accuracy of predicting such *post-mortem* traits in primal cuts or retail cuts using CT of the meat cuts themselves (rather than live animal CT) and multi-object image analysis (Appendix i) which would reduce the cost of CT scanning individual cuts by allowing several objects to be scanned and analysed simultaneously.

7.2.2 Intramuscular fat

Following a review of the literature by Savell and Cross (1988) a minimum level of 3% IMF in grilled cuts of red meat such as beef and lamb was recommended to ensure consumer

acceptability in terms of eating quality, with some studies recommending as high as 5% (Hopkins et al., 2006) in lamb meat, which we may define as a minimum 'window' of acceptability ranging from 3-5% IMF in lamb. Although it should be noted that a current and comprehensive study on the relationship between IMF in lamb and consumer taste panel results in the UK has yet to be carried out and previous studies have highlighted country differences in preferences of lamb meat (Sanudo et al., 1998).

This study provides evidence that in both the experimental animals included in chapter two and the commercial animals included in chapter six, the average levels of IMF within both populations falls well below these recommended levels for optimal eating quality (Chapter 2: Mean IMF = 1.48%, Chapter 6: Mean CT predicted IMF% = 1.84 – 2.32% dependant on prediction model). These initial findings reinforce the requirement for increased attention to levels of IMF in the production of lamb meat in the UK. This is of course restricted by current methods of determining IMF levels *post-mortem*. However both chapters two and three have provided evidence of the ability of CT to predict with high accuracy IMF content in the loin of Texel sheep *in-vivo*. The methods used during the analyses were intended to be thorough in the process of including a large number of possible combinations of CT measures available, alternative image processing, and also using both two-dimensional, three-dimensional and a combination of these CT methods. This approach succeeded in identifying optimum prediction equations balanced for accuracy and practical application amongst all possible combinations of CT measures and methods, providing robust and accurate estimations of IMF content in Texel lamb loins. Throughout the study, it was considered that, the use of CT predicted IMF, where the prediction included total carcass fat, may complicate the divergent genetic selection for increased IMF against a reduction in carcass fatness. Therefore it has been attempted to build and use prediction models with a higher independence of CT predicted carcass fatness. To address this prediction models were

developed both including and excluding related measures of total carcass fatness and the method of virtual dissection has been used.

The results from chapters two and three identified several possible prediction models for IMF producing prediction accuracies ranging from 63% - 71%, greatly improving on accuracies reported in previous studies ranging from R^2 0.36 – 0.57 (Karamichou et al., 2006; Lambe et al., 2010a; Macfarlane et al., 2005). No significant improvement was made on the accuracies achieved from virtual dissection, the use of three-dimensional CT measures or the combination of both two-dimensional and three-dimensional CT measures. The selection of optimal models was based on the use of CT information from current commercial CT methods using two-dimensional CT measures from three available reference scan images, three-dimensional spiral CT information and a combination of two-dimensional and three-dimensional. And for the reasons previously highlighted two optimal models were chosen; one including and another excluding CT predicted total carcass fat weight. As we know that there is a scanner effect on density values within soft tissue ranges between different scanners (Bunger et al., 2010), and two different scanner types were used between farms in both the experimental and commercial scanning procedures, scanner-specific equations were developed. Two models were ultimately selected from the work carried out in chapters two and three.

7.2.3 Breed and sex effects on IMF and the application of CT predicted IMF models in different breed types

The prediction equations developed in chapter two on Texel sheep were applied across divergent breed types for which CT and chemical IMF data were available (Texel, Scottish Blackface and Texel cross Scotch Mule), the purpose of which was to investigate the accuracy of transferring CT prediction models developed on one particular breed type to other breed types. The IMF levels across the breed types and sexes were also compared.

The Texel population included in the study were significantly lower in both IMF and CT predicted carcass fat than Scottish Blackface and Texel cross Mule sheep. And in the same animals this has translated to increased tenderness, stronger lamb flavour and higher overall liking in the Scottish Blackface when compared to the Texel lamb meat (Navajas et al., 2008), further affirming the effect IMF levels play in the perception of organoleptic traits such as tenderness, flavour and overall liking. Scottish Blackface lambs were on average highest in both carcass fat and IMF, followed by Texel cross Mule and finally Texel. Again this highlights the effect of breeding strategies focussed on increasing lean meat production while maintaining or reducing overall carcass fatness in terminal sire breeds when compared to breeds which do not focus so much on the terminal traits e.g. Scottish Blackface. That is not to suggest that the inclusion of CT MQ and carcass traits in maternal and crossbreeding selection programmes would not be of interest (Conington et al., 2006) and chapter four provides evidence that CT predicted IMF may be applied directly to Scottish Blackface.

Males on average were leaner across all breeds when compared at the same liveweight, which agrees with several studies reporting that entire males are on average the leanest, followed by castrates and females (Bass et al., 1990; Butler-Hogg et al., 1984; Dransfield et al., 1990; Kirton et al., 1982). In this study, males also showed lower levels of IMF at the same levels of carcass fatness, with the exception of the Texel cross Mule lambs which were shown to have similar mean levels of IMF to their female counterparts, which is no doubt a reflection that these were castrated males and the Texel and Scottish Blackface males were entire.

It was expected, that given the breed relationship between Texel and Texel cross Mule, the prediction equations would transfer across better than to the Scottish Blackface. However, it was the opposite, prediction accuracies in the Scottish Blackface data ranged from R^2 0.57 – 0.64 (RMSEP 0.49 – 0.54) and in the Texel cross Mule data accuracies ranged from R^2 0.36 – 0.37 (RMSEP 0.48 – 0.49). To investigate the differences in transferability across the

breeds, obvious differences in the summary statistics were highlighted, with differences in age at CT and age at slaughter identified across the breed types. The further investigation of these age related differences and their effect on transferability, provided evidence that there was no effect of age either at CT or slaughter on the prediction accuracies. Furthermore, a breed specific approach was also taken to the Texel cross Mule dataset, producing breed specific coefficients for the same models developed in chapter two. This also resulted in no improvements of accuracies. It was shown in chapter four that there are differences in the relationship between IMF and CT variables across the breed types, which may explain the reduction in accuracies of prediction equations developed in Texel across to the Texel cross Mule. This suggests that breed specific equations may be required rather than simply breed specific coefficients. It should also be acknowledged that the structure, design and the experimental procedures of the experiments providing the data was not optimal for a definitive and comprehensive breed comparison for both IMF levels and the prediction equations. A structured study balancing fixed effects such as sex, breed type and random effects such as age at CT, age at slaughter, management regime etc. would be recommended to produce thorough, definitive and comprehensive results. These results are indicative of the transferability of the prediction equations developed in chapter two, however it would be recommended, that if these prediction equations were to be considered in other breed types, validation studies should be conducted to confirm the accuracies achieved.

7.2.4 Genetic parameters of ultrasound, CT estimated and meat quality traits in Texel sheep

There were ultimately two parts to the genetic analysis in the study from chapters five and six, firstly the estimation of genetic parameters using the same, size limited research data set as used in chapters two through to four, and secondly the use of a larger, more powerful dataset comprising historical commercial data held within the BASCO database. The initial genetic analyses using the research data had the aim to produce genetic relationships

between *post-mortem* meat quality measurements such as shearforce and IMF and CT estimated traits including the novel CT predicted IMF traits. It was discovered that a combination of small animal numbers, and the design and research objective of the study to produce some of the data, resulted in a pedigree structure that limits the effectiveness of the research data set for genetic parameter estimation, this was as a result of both males and females being intensively selected in order to increase the genetic frequency of a QTL that was of interest for that original study. Closely related individuals in part of the study were used as parents and the common sires could be traced back to a single sire. Therefore, the aims of the initial genetic analysis were very difficult to achieve and results reported were seemingly unreasonable with regards to magnitude and /or accompanied by very large standard errors. The primary interest in this chapter was the quantification of the genetic basis and relationships between *post-mortem*, laboratory measured traits, with *in-vivo* meat and carcass quality traits. This remains a valuable relationship to understand and would require large numbers of animals, including pedigree information, CT data and laboratory measured MQ traits to achieve this. The CT methods of IMF prediction developed in this study may serve to enable the robust genetic analysis of these traits in future research or commercial studies.

Robust and accurate heritability estimates of the novel CT predicted IMF traits and genetic correlations with existing index traits are the main focus, alongside the confirmation of genetic parameters of current US, CT and growth traits in order to enable the inclusion of CT predicted IMF into current breeding programmes. A larger industry data set was made available from the BASCO database, making it possible to estimate genetic parameters of these economically important traits. Moderate heritabilities were estimated for growth traits, with moderate to high heritabilities estimated for US and CT traits. Heritability estimates for the novel CT predicted IMF traits were moderate (h^2 0.31-0.36) and strong positive genetic correlations were estimated between US measured fat depth and CT predicted IMF (r_g 0.60-

0.64). Of particular interest was the genetic relationship between CT predicted fat weight and CT predicted IMF using each of the two models: which was found to be strong and positive for the model inclusive of CT predicted carcass fat weight (r_g 0.83) and moderately positive for the model entirely independent of CT carcass fat measures (r_g 0.59). The genetic relationship between the two CT predicted IMF traits were strong and positive (r_g 0.89).

The heritability estimates for CT predicted IMF produced were similar to those for chemically extracted IMF found in previous studies (h^2 0.32-0.48; (Karamichou et al., 2006; Lorentzen and Vangen, 2012; Mortimer et al., 2014). The similarity between heritabilities for CT predicted IMF and IMF is an indication of the prediction accuracy of CT predicted IMF *in-vivo* in Texel sheep. It is also apparent that both models are partially under different genetic control from CT carcass fatness. However the model not inclusive of CT carcass fat measures was less genetically correlated to CT carcass fat than the model inclusive of these measures. This provides evidence that a model for prediction of IMF that is not using carcass fat as a predictor can provide a similar accuracy as a model that uses CT carcass fat measures. Any selection scheme using CT to improve or maintain IMF and to reduce further carcass fat, works against the positive genetic correlation between the two fat depots. An IMF prediction model that is not using the information on carcass fat as predictor should be very valuable in such an approach aiming to identify "correlation breakers" as selection candidates, given that there is a large amount of variation in both fat depots (carcass fat and CT predicted IMF) in the commercial population used here (Figure 7.1).

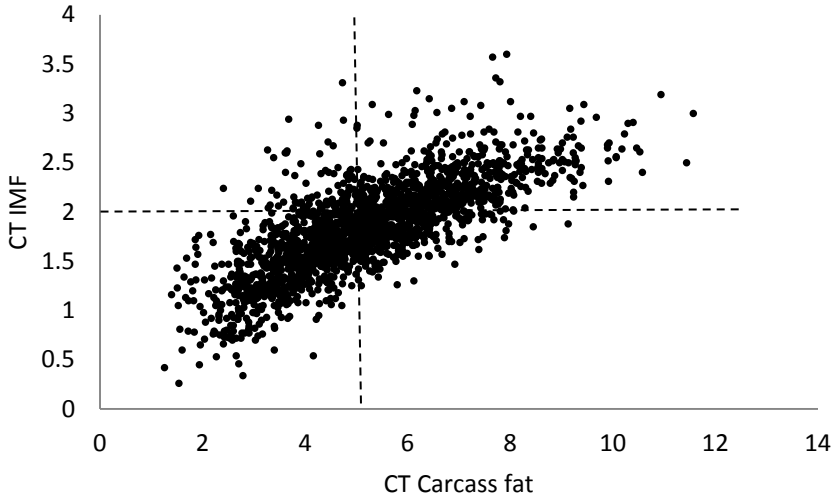


Figure 7.1: Plot of selection candidates from commercial CT data based on CT predicted carcass fat and CT predicted IMF using model PIMF2 (chapter 5)

There may already be potential selection candidates within the commercial population which fit the criteria for lean carcasses at optimal levels of IMF, such as those within the top left quadrant in Figure 7.1. It can be concluded that CT provides a highly accurate tool to identify these selection candidates.

Currently the Texel breeding programme's multi-trait selection index focusses mainly on increased US muscle depth, CT muscle weight and a very slight reduction of CT carcass fat weight. Estimated breeding values for IMF should be immediately introduced into current breeding programmes for Texel sheep and in the future other breeds. Given that the Texel breed is already very lean in comparison to some other breeds as discussed previously, the inclusion of CT predicted IMF into the existing multi-trait selection index would enable breeders to maintain the lean and muscular attributes of the breed whilst selecting for increased IMF levels which are closer to the levels recommended for optimal eating quality, providing the industry with an improved balance of economically important carcass quality traits in the abattoir alongside optimal eating quality characteristics for the consumer.

It is of note in this context, that future value based payments systems in abattoirs will probably reward farmers for meat quality and the time unit of breeding is generations not weeks or months. In other words, such an approach would make the industry more future proof.

7.3 Future work

Further steps to achieve the integration of these CT predicted IMF methods into current two-stage selection practices and routine genetic evaluations would require the addition of CT predicted IMF into current commercial analyses of CT images alongside the integration of CT predicted IMF into current multi-trait selection indices and the existing two-stage selection programmes for Texel sheep. The investigation and further development of CT predicted IMF methods in other terminal and maternal breeds should be continued and will lead to additional benefits of CT to the entire UK sheep breeding industry.

One area of potential further research related to the eating quality of lamb, is the prediction of eating quality in primal or retail cuts of meat. Very high accuracies have been achieved in beef primal cuts (Prieto et al., 2010) but not yet in lamb loins (Appendix i). However a more structured and thorough analyses of several types of cuts, including primal cuts such as the entire saddle, gigot and shoulder of lamb down to rib joints and fully dissected loins may be more successful in the quantification of meat eating quality traits in lamb from CT scanning meat cuts. These suggested subsequent studies should also further investigate the effect of in vivo CT scanning vs. *post mortem* with the latter possibly affected by chilling or even freezing and thawing.

7.4 Conclusion

This thesis has provided robust evidence that CT can be used as a highly accurate method of predicting IMF as a proxy trait for meat eating quality in live Texel sheep and potentially

other breeds, and has the potential to be incorporated into the current two-stage selection approach and the routine genetic evaluation of Texel sheep in the UK. The inclusion of CT predicted IMF will undoubtedly add further value to the benefits of CT scanning and could assist in the production of optimal eating quality lamb meat, whilst maintaining or further improving important carcass attributes.

Appendix i: Use of computer tomography (CT) to predict chemical intramuscular fat (IMF) in dissected lamb loins

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Value for Industry

- The prediction of IMF during processing of retail cuts in a non-destructive manner, independent of any fixed effects.
- The potential to provide a valuable commercial tool to maximise levels of eating quality and the foresight to target certain markets.
- Provide information on meat quality to feed into breeding programmes
- Non-destructive prediction of eating quality which could provide information as a basis to reward commercial farmers for meat quality

Background

In different livestock species, meat eating quality (MEQ) traits such as flavour, tenderness and juiciness are known to be linked to fat levels. This association of MEQ attributes and fat levels in meat are largely due to the positive association with intramuscular fat (IMF) (Savell and Cross, 1988). X-ray computed tomography (CT) can measure total fat, muscle and bone *in vivo* in sheep and CT predictions of carcass composition have been used in commercial

UK sheep breeding programmes over the last two decades (Bunger et al., 2011). Together with ultrasound measures of fat and muscle depth in the loin region, CT measured carcass fat and muscle weights have contributed much to the success of breeding for leaner carcasses and led to higher selection responses (Moore et al., 2011).

Previous research has not only demonstrated that *in vivo* CT can predict or measure total carcass fat, but it can also provide measurements of the average muscle density, which has been shown to be a good predictor of IMF (Karamichou et al., 2006; Young et al., 2001). The application of CT technology in primal or retail cuts of lamb has not been investigated as thoroughly. The use of spiral CT scanning (SCTS) and the development of multi-object scanning may provide additional advances in the application of such technology at the time of processing. Multi-object spiral CT scanning provides high resolution CT images in a cost effective way, generating 3D images and precise measurements of tissue volumes and weights of several objects at the same time. Although there is continued investigation into the prediction of meat quality traits in lamb using *in vivo* CT, using both information from reference scanning and 3D spiral scanning, relatively few studies have focussed on primal or retail cuts.

Why work is needed

The provision of meat quality assurances from meat processors is certainly of interest to both the consumer and producer. For the consumer, an assurance of certain minimum levels of meat quality traits should increase confidence at point of purchase and omit requirement for visual evaluation by consumers. For the producer, knowledge of meat quality aspects may be fed into breeding programmes and future payment systems would have relevant information to draw from to reward producers on meat quality. All these aspects would enable the processor to target certain markets and better evaluate markets and consumer preferences.

The ability to measure lamb meat quality in a non-destructive and safe manner, maintaining the integrity of the product, is important for any such system to be applied in industry. The aim of this experiment was to investigate the ability of CT to predict intramuscular fat (IMF) in a retail processed cut of meat (*M. longissimus*) using only CT derived information.

The methods used

Two hundred and two rear right side lamb loins (Lumbar vertebra 1-7) were removed post-slaughter, vacuum packed, chilled and aged for seven days on the bone. After this time the bone was removed, and the loin's were re-packaged and frozen for transportation to the SRUC/BioSS CT unit, Edinburgh, UK for CT scanning.

The front portion of these same 202 loins (thoracic vertebra region) were split into three separate pieces 3 days post-slaughter, immediately vacuum packed and frozen for further analytical testing, as part of the wider study, including IMF estimation using the direct bi-methylation method described by Lee *et al*. (2012). The section of the loin that included the last rib (14^{th} thoracic vertebra) was thawed for 14 hours, visible fat and connective tissue were removed and $1cm^3$ samples were cut. Chemical IMF (chem_IMF) was calculated as the sum of the major fatty acids in a fatty acid profile (mg/100g fresh weight).

The rear right loins (lumbar vertebra region), which were transported frozen to the SRUC/BioSS CT unit in Edinburgh, were thawed over a period of approximately 12 hours under refrigeration, over a 10–day period, in six batches of 30 samples and one batch of 22 samples. Loins were uniformly orientated and positioned on a multiplex scanning frame and spiral CT scanned (contiguous scans at 8mm intervals) in batches of six (Figure xvii). Each batch was scanned at two separate x-ray intensities (80kV and 130kV). The purpose of this was to investigate whether images produced at 80kV would increase the contrast of voxels within the soft tissue, compared to the standard 130kV intensity, and therefore improve on

the accuracies of prediction in IMF following a similar approach by Stubjkjaer-Schubert *et al.* (2009).

Figure i: Dissected loins orientated and positioned in the multiplex frame (left), 3D rendered image of multiplex scanning (right)

CT images were segmented using a multi-object animal tomograph analysis routine (ATAR) developed at BioSS/SRUC (Figure xviii). Each pixel in each image was allocated as fat or muscle, using previously developed density thresholds, specific to the analysis of images obtained from carcasses, primal cuts and dissected muscles (fat = -244 to 24 HU, muscle = 26 to 204 HU), which were different to the thresholds used for live animal scanning. The CT density results were then weighted by area in each image and averaged over the spiral series images (26-30 images per loin, average = 28 images).

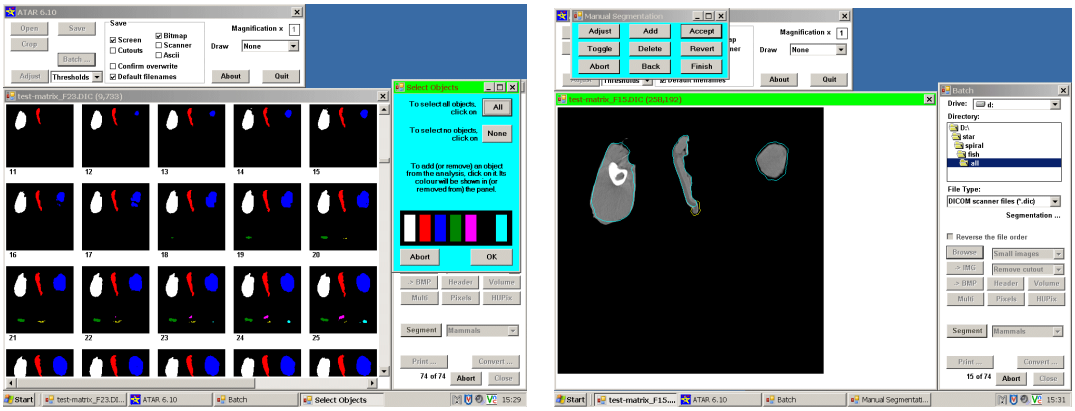

Figure i: Selection of the scanned objects using ATAR software (left), adjusting the boundary of a selected object (right)

To predict the chem_IMF from CT measured traits, the statistical analysis included the weighted CT density information from each spiral loin scan, this included: loin weight at CT scanning (Ct_wt) calculated from the CT derived volume and weight (g/cm^3) of the soft tissue (muscle and fat); weighted muscle and fat densities (w_md, w_fd) and their standard deviations (w_msd and w_fsd); weighted soft tissue densities (combining the density ranges between fat and muscle) and their standard deviations (w_std, w_stsd); and the proportion of voxels allocated as fat (Pr_F_vox). Nine models were tested in the analysis, using different combinations of CT variables in each maximum model. Models containing three or more variables were analysed using generalized stepwise linear regression in Genstat14 (Payne et al., 2012) optimising the use of predictor variables within the more complicated models, while simpler models containing a maximum of two predictor variables were analysed using multiple linear regression. Models were then tested for significant differences using their correlation coefficient ($\sqrt{\text{Adj } R^2}$) and applying standard methods.

The results obtained

Chem_IMF% ranged from 1.27% to 4.71% with a mean of 2.49% and a coefficient of variation of 22.94.

Table ii shows the regression parameters (Adj R^2 and RMSE) of the predictive models obtained from multiple and generalised stepwise linear regression analysis from the maximum models tested. Using CT calculated loin weight and muscle density at an intensity of 80kv resulted in very poor prediction of IMF% (Adj R^2 0.04; model A), with similar poor results using fat density and combining muscle and fat density (Adj R^2 0.06; models B and C). The use of soft tissue density and its standard deviation resulted in similar prediction accuracies (Adj R^2 0.04, 0.05; models G and H). However, the use of CT calculated loin weight, average muscle density across the loin and the standard deviation of muscle density resulted in a significant increase in accuracy (Adj R^2 0.18; model D). A further slight increase in accuracy (Adj R^2 0.20; model I) was achieved when the proportion of voxels allocated as fat was included in the model. No significant improvement was found using results obtained from the higher CT intensity (130kv). The maximum accuracy of prediction achieved was an Adj R^2 of 0.14 at 130kv.

Table i: Linear regression models between chem_IMF and CT tissue parameters, with adjusted coefficient of determination (Adj R2) and residual mean square error (RMSE)

		80kV		130kV	
	Maximum Model	Adj R^2	RMSE	Adj R^2	RMSE
A	Ct_wt+w_md	0.04	0.56	0.04	0.56
B	Ct_wt+w_fd	0.06	0.56	0.07	0.55
C	Ct_wt+w_md+w_fd	0.06	0.56	0.07	0.55
D	Ct_wt+w_md+w_msd	0.18*	0.52	0.13*	0.53
E	Ct_wt+w_fd+w_fsd	0.06	0.55	0.07	0.55
F	Ct_wt+w_md+w_msd+w_fd+w_fsd	0.18*	0.52	0.14*	0.53
G	Ct_wt+w_std	0.04	0.56	0.04	0.56
H	Ct_wt+w_std+w_stsd	0.05	0.56	0.05	0.56
I	Ct_wt+w_md+w_msd+Pr_F_vox	0.20*	0.51	0.14*	0.53

*√Adj R^2 are not significantly different when tested using Fisher's Z transformation

The scientific conclusions

In this study, CT was unable to predict IMF content of dissected lamb loins with acceptable accuracy. However more promising prediction accuracies (R^2 0.33 - 0.44) have been reported in previous studies using a small sample of 30 dissected loins (Lambe et al., 2010b). In the same study, prediction accuracies varied when using different scanners (R^2 0.05 – 0.12). Live animal assessments use entire carcass images (including other fat depots such as subcutaneous and intermuscular fat), and therefore the poorer results obtained during the current study may suggest that there is additional information within these other carcass portions and fat depots which provide increased accuracies in prediction of IMF. Another factor may be that the composition of the muscle *in vivo* and post processing changes, in terms of the density of soft tissue, which results in the reduction of prediction ability when using density measures of processed retail cuts of meat. This may be further supported by substantially improved prediction accuracies during a separate study where virtually dissected loins from live animal scans resulted in accuracies of 62% (Clelland et al.Unpublished)

The next steps

Further research will be carried out investigating the prediction of IMF in primal cuts of lamb, including additional fat depots (subcutaneous and intermuscular) to determine the role that these fat depots may play in the accurate prediction of IMF in retail or primal cuts. Comparative data sets are available to investigate the difference in accuracy when using virtually-dissected images from live animal scanning to isolate tissue included in both retail and primal cuts, alongside CT scan data from butchered primal cuts and fully dissected loins.

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