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Spine characteristics in sheep: Metrology, relationship to meat yield and their genetic parameters

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Abstract

The overall accuracy, efficiency and profitability of livestock improvement strategies can be greatly increased by incorporating quantitative genetics into livestock selection and breeding. Since the introduction of quantitative genetics, a range of traits describing the animal e.g. in terms of health, growth, fecundity, production, have been extensively evaluated in terms of genetics and are now commonly manipulated through breeding to achieve specific selection goals.

An industry led enquiry as to the possibility of including spine traits in genetic selection to increase back length in sheep was the basis of the present thesis. Collecting information on spine traits (spine length, vertebrae length and vertebrae number) is of particular interest and use to the sheep breeding industry as there may be the potential to increase meat yield from the highly valuable *longissimus thoracis et lumborum* (LTL or loin), located parallel to the spine, with little associated change in production costs.

The thesis focusses on the use of X-ray computed tomography (CT) scanning as a technique which would allow spine traits to be measured *in vivo*, hence being useful for genetic selection. The topogram scans produced from the CT scanning procedure were analysed to derive spine trait information for the thesis. The scans were from Scottish Blackface (maternal breed stock), Texel (terminal sire breed), Texel cross Mule and Poll Dorset cross Mule (three-way cross slaughter lambs) so as to represent the divergent genotypes found across the different levels of the United Kingdom's (UK) three-tier crossbreeding structure of sheep.

The present study explored as a first step intra- and inter-operator repeatability of assessment of spine traits from CT derived topograms, as a means to investigate the suitability of the approach for widespread uptake within industry where operators will vary. The results showed that there was high repeatability for intra- and inter-operator assessment of spine trait measurements verifying that the CT method could be accepted as a reliable alternative (to slaughter for example) to quantify spine traits.

To determine whether spine traits are similar across the range of breeds representing the key genotypes and crosses in the UK sheep industry, numerous CT topograms were analysed. The results showed marked variation in spine traits within and between Scottish Blackface, Texel, Texel cross Mule and Poll Dorset cross Mule breeds and crosses. For example, the Texel breed

was found to have the largest within-breed range for thoracolumbar vertebrae number (17 – 21; the majority possessing 19), but the spine length of these animals was, on average, significantly shorter than the other breed/cross groups. The present study concluded that the significant differences between the breeds and breed types for the particular spine traits were possibly indicative of a genetic control for these traits.

Furthermore, investigation into the phenotypic correlations between spine and production traits revealed some directional associations which may prove beneficial for meat production. For example, Scottish Blackface lambs which had a longer length of a specific spine region had an associated decrease in the volume of carcass fat. Texel lambs which had a longer length of a specific spine region had a slightly larger loin muscle area, at a given weight.

The present study also examined animals from a population of Texel lambs already heavily selected for increased muscling. The Texel muscling quantitative trait locus (TM-QTL), segregating in these animals and generally in the UK's Texel sheep population, is expressed through a polar overdominance pattern of inheritance and its effect on the loin (localised muscle hypertrophy) is commonly utilised in the selection and breeding of Texel sheep to improve meat production. Examination of topograms from lambs bearing the whole range of TM-QTL genotypes showed little evidence to suggest that the change in loin shape/increased loin muscling, as a result of the TM-QTL and its inheritance, has led to any associated change in the underlying spine characteristics. This suggests that selection for increased muscling associated with the TM-QTL may be achieved independently of changes in the spine traits studied.

The potential to breed for certain spine traits to increase vertebrae number and hence chops or loin yield can be enhanced by establishing the genetic parameters for the traits. The present study employed a collection of performance trait records from Texel lambs to provide the basis for genetic analysis. The results showed different levels of heritability for the different spine traits but also high standard errors. For example, heritability of vertebrae number was dependent on vertebra location: for thoracic vertebrae heritability was high (h^2 = 0.99; SE = 0.42), for lumbar vertebrae heritability was low (h^2 = 0.08; SE = 0.12), whereas in contrast, thoracolumbar vertebrae heritability was moderate (h^2 = 0.44; SE = 0.27). Phenotypic and genetic correlations between all combinations of traits were also obtained.

Accurate predictions of the size and direction of response to selection can be achieved through such genetic analysis of traits. The more that is known of the genetic characteristics of traits and their genetic correlations with other economically important traits, the more efficiently it can be built into breeding programmes improving the overall performance of stock. The results of this study showed that providing spine measurements can contribute to the diversity of trait information available to breeders. The present study also suggests that there may be opportunities to select for increased spine length/vertebrae number which would benefit the sheep industry in terms of increased chop number/loin yield. Although more data are needed prior to implementation. Practical uptake of selection for spine traits would be enhanced due to the straightforward nature of the measurements and the high operator repeatability.

Declaration

I declare that I have composed this thesis. The research contained within is my own work and any assistance has been acknowledged. The work has not been submitted for any other degree or professional qualification.

Claire L. Donaldson
October 2015

Publications in peer reviewed journals

Donaldson, C.L., Lambe, N.R., Maltin, C.A., Knott, S. and Bunger, L., 2013. Between- and within-breed variations of spine characteristics in sheep. *Journal of Animal Science*. 91, pp.995-1004.

Donaldson, C.L., Lambe, N.R., Maltin, C.A., Knott, S. and Bunger, L., 2014. Effect of the Texel muscling QTL (TM-QTL) on spine characteristics in purebred Texel lambs. *Small Ruminant Research*. 117, pp.34-40.

Other publications

Donaldson, C.L., 2011. Quantifying spine characteristics in sheep using computed tomography. *Quality Meat Scotland, Research and Development 2010/11*. pp.24-25.

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Now, to my family, you didn't let me give up. Thank you for that and thank you for your continued support in so many ways that meant I didn't have to. I know you didn't have much of a clue about what my project was about but now you can enjoy reading about it.

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Chapter 1

General introduction

1.1 The sheep industry

1.1.1 Position of the United Kingdom's sheep industry within the European Union

The European Union (with 28 Member States; EU-28) is considered a world leader in the production of red meat along with several other products (vegetables, cereals, dairy products etc.). To this, the United Kingdom (UK) contributes significantly and performs particularly well with regards to production of sheep (lamb and mutton) meat. From the most recent data available for Europe (European Commission, Eurostat, 2014) regarding (i) total number of sheep and (ii) sheep meat production, the approximate situation of the UK's sheep industry may be described and its position amongst the European Union Member States recognised. Figure 1.1(a), for example, illustrates that in the EU, the UK possessed the largest stock of sheep (22,624 thousand) in (December) 2013 and in turn was the largest producer of sheep meat for the same year (Figure 1.1(b)). Furthermore, with a female breeding flock of 14.8 million in size in 2013, which was ~23% of the EU's total female breeding flock (European Commission, Eurostat, 2014), it places the UK in a position where it can maintain such sizable production and slaughter levels.

The EU-28 plays a major part in world trade as the largest importer and second largest exporter of all types of agricultural products. With respect to the 2013 sheep meat export levels, the UK was a key contributor to the EU-28, moving a volume of ~103,000 tonnes (includes exports to non-EU and EU countries); a 9% rise from 2012 (EBLEX, 2014a). This is a significant increase, and if maintained, opens the opportunity for the country to move into a stable position in the consumer marketplace where competitive prices can be offered and overall financial returns increased. As it stands, the UK sheep industry excelled in the production, processing and exportation of sheep meat in 2013. It is apparent that the UK has an extremely proficient sheep industry with a somewhat unique breeding structure which has helped the country secure this position.

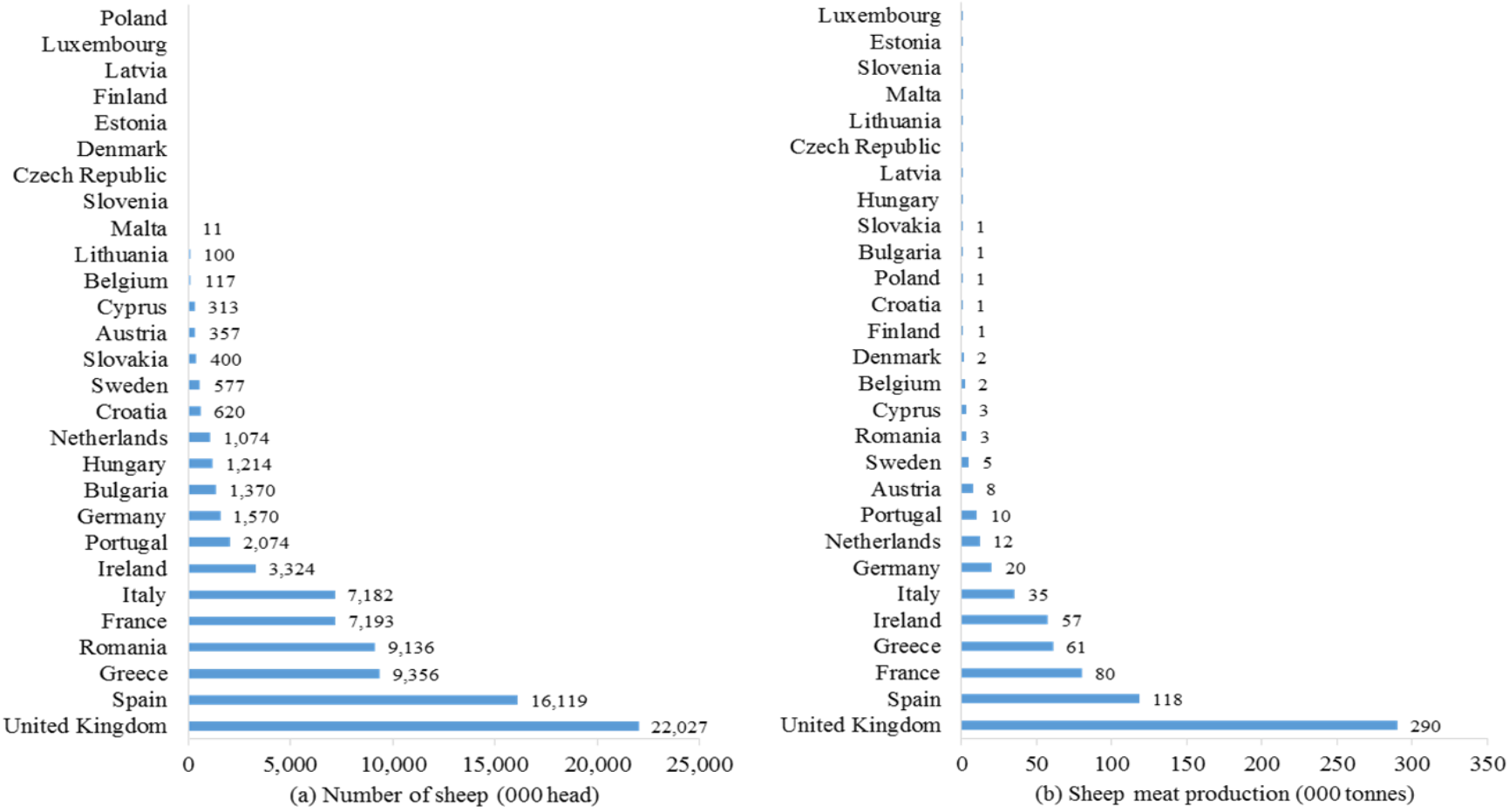


Figure 1.1 Charts illustrating the number of sheep recorded (in December) (a) and volume of sheep meat produced (b) in each European Union Member State in 2013. *Source: European Commission, Eurostat, 2014*.

1.1.2 The United Kingdom's three-tier crossbreeding structure

Sheep are multipurpose, small ruminant animals providing a source of milk, meat and wool, so for many countries of the EU the sheep industry plays a key role in their economy (Pollot and Stone, 2006). Sheep are diverse in terms of both the number of recognised breeds and crosses and the environments to which they have become adapted. This makes them ideal in exploiting a wider range of secluded, harsh and poorer conditioned areas or pastures that prove unfavourable or unsuitable for other agricultural purposes (Pirisi et al., 2007; Sargison, 2008).

Historically, the main reason for keeping sheep flocks was to breed and rear the animals for wool and, on a lesser scale, milk; sheep meat was classed as the by-product (Boutonnet, 1999). Now, low returns on wool production and the increasing use of synthetic materials, which have improved in price, quality and processing (Boutonnet, 1999; Meat Trade News Daily, 2010), have left very few EU countries active in wool production, manufacturing or trade. Similarly, only a small number of European countries (Greece, Spain, France and Italy) use their sheep industry for milk production and this too tends to only be on a local scale (Pirisi et al., 2007). The primary function of the UK sheep industry has become meat production, particularly lamb *"the only final product from the UK sheep industry of any significance is lamb meat"* (Pollott and Stone, 2006). With the industry favouring meat production, the UK has become a key player in the European (Figure 1.1(a) and (b)) and the world sheep meat market; milk and wool are regarded as minor outputs in the UK.

The breeding structure adopted in the UK has been critical to the maintenance of the UK's top position in sheep meat production, processing and exportation. Characterised by a stratified three-tier crossbreeding system it is not like any other presently used. It has evolved over the years; matching the numerous breeds and crosses to different production systems and structures to best utilise the available land (Macfarlane and Simm, 2008). A brief summary of this structure follows, aided by Figure 1.2, however, more detailed descriptions are provided by Pullar (2003) and Pollot and Stone (2006).

On the whole, the stratified three-tier crossbreeding system places the majority of emphasis on the production of better lambs for slaughter. Pure bred hill ewes are mated pure or with pure bred longwool rams; the latter produces crossbred ewes, known as Mules, which are largely put to sale. Typically in Scotland, the hill ewe breed is the Scottish Blackface (Figure 1.2(a)) and the longwool crossing rams are Bluefaced Leicester (Figure 1.2(b)); the Mule ewes of the resultant cross are known as Scotch Mules (Figure 1.2(c)). The Mule ewes, known for

their prolificacy and maternal traits, are mated to rams of terminal sire breeds, mainly Texel (Figure 1.2(d)) and Suffolk (Figure 1.2(e)), (though the use of Beltex and Charollais are increasing) which tend to be bred more intensively for factors such as growth, size and carcass traits (Pullar, 2003; Macfarlane and Simm, 2008). Figures for 2003 indicated that 71% of all of the three-way cross slaughter lambs (example, Figure 1.2(f)) produced in Britain were sired by rams belonging to a terminal breed (Pollot and Stone, 2006), the majority of which go directly into the meat production chain. This reflects the importance of these terminal breeds in the UK and highlights the overall aim for their use in the crossbreeding structure, the production of heavier finished lambs with improved carcass quality for the sheep meat market.

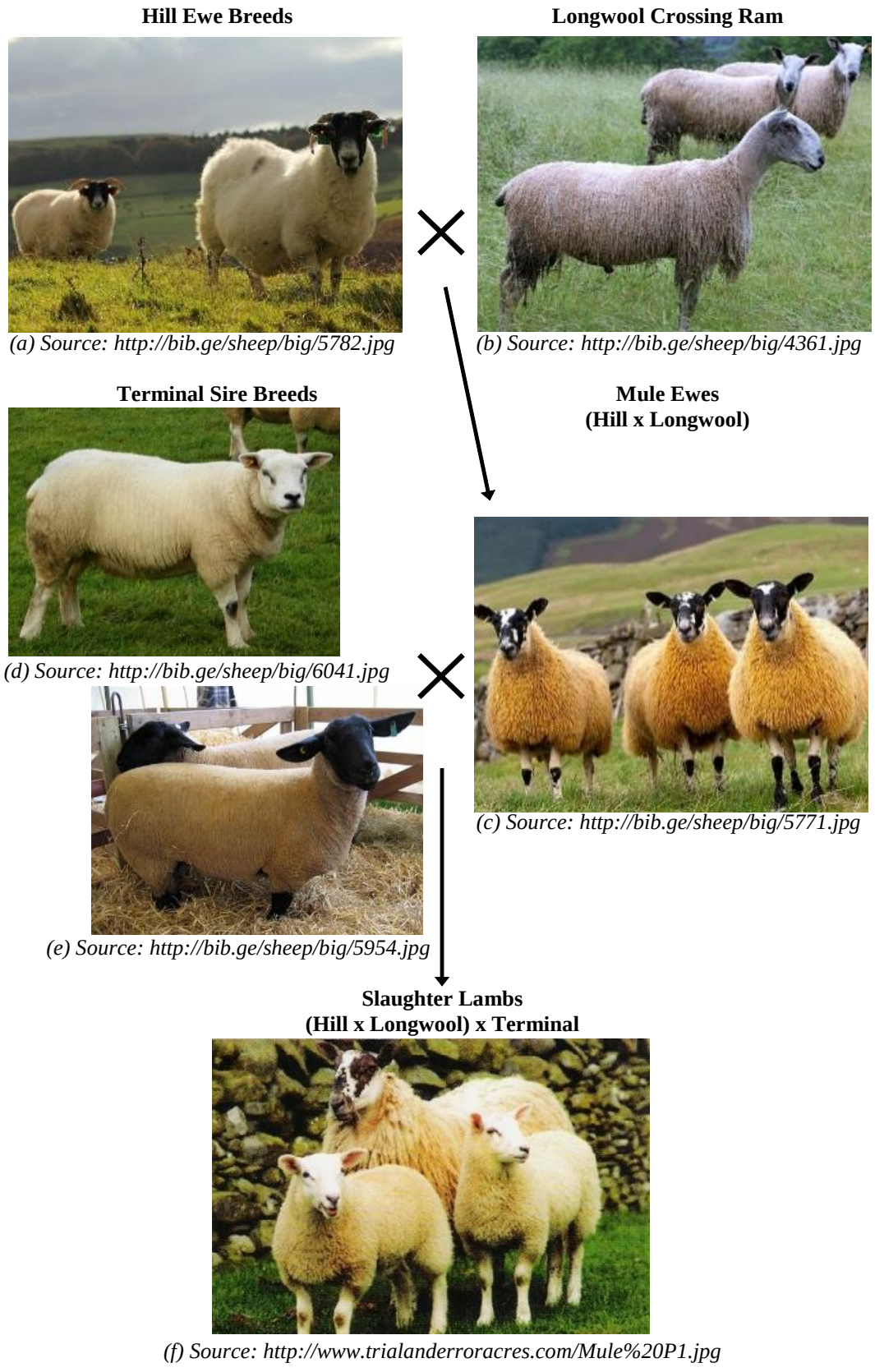


Figure 1.2 Summary diagram of the three-tier crossbreeding structure of sheep in the UK.

1.1.3 The trend of the United Kingdom's sheep stock numbers

Although meat production is the highest priority in the UK sheep industry, over the last decade meat production levels were much lower compared to what was produced 15-20 years ago in the UK (Figure1.3). It may be proposed that the yearly change in the total number of sheep (ewes intended for breeding and/or slaughter, rams and other sheep one year and over) and lambs (sheep under one year old) held within the UK stock (Figure 1.3) has been a primary factor contributing to the fluctuations in production levels.

From the 1970s leading into the early 1980s, the number of sheep kept in UK flocks was in steady growth. Over the period 1987 to 1999 numbers peaked with an average of ~43.3 million sheep recorded and an average of ~350 thousand tonnes of meat produced each year (Figure 1.3). A noticeably sharp decline in sheep numbers from 1999 to 2001, where ~42.2 million sheep and lambs dropped to ~36.7 million, was mainly due to an outbreak of Foot and Mouth Disease (FMD) and in the interests of food security it was a requirement that many thousands of animals were culled. Understandably, this was reflected in sheep meat production levels with 259 thousand tonnes of sheep meat produced that year, the lowest levels recorded over the period 1985 to 2013 (Figure 1.3).

Following from 2001 into 2010 there was still further decline in sheep numbers in each year. A final revision to the estimates for UK sheep and lamb numbers for (June) 2010 was recorded at 31.1 million. Despite this, meat production levels did start to recover from 2001 to 2010 and remained relatively stable, however, the industry was still not quite producing the same volume of sheep meat that was achieved in many of the years across the late 1980s and through into the 1990s. Through years 2011, 2012 and 2013 a decrease in sheep meat production occurred even though the growth in the numbers of sheep and lambs had improved. However, this has even been forecasted to halt given difficult and fluctuating conditions (e.g. seasonal weather extremes, high culling rates) experienced over the 2012/13 period (EBLEX, 2013a, 2014b), making the future situation of sheep and lamb numbers and meat production difficult to forecast.

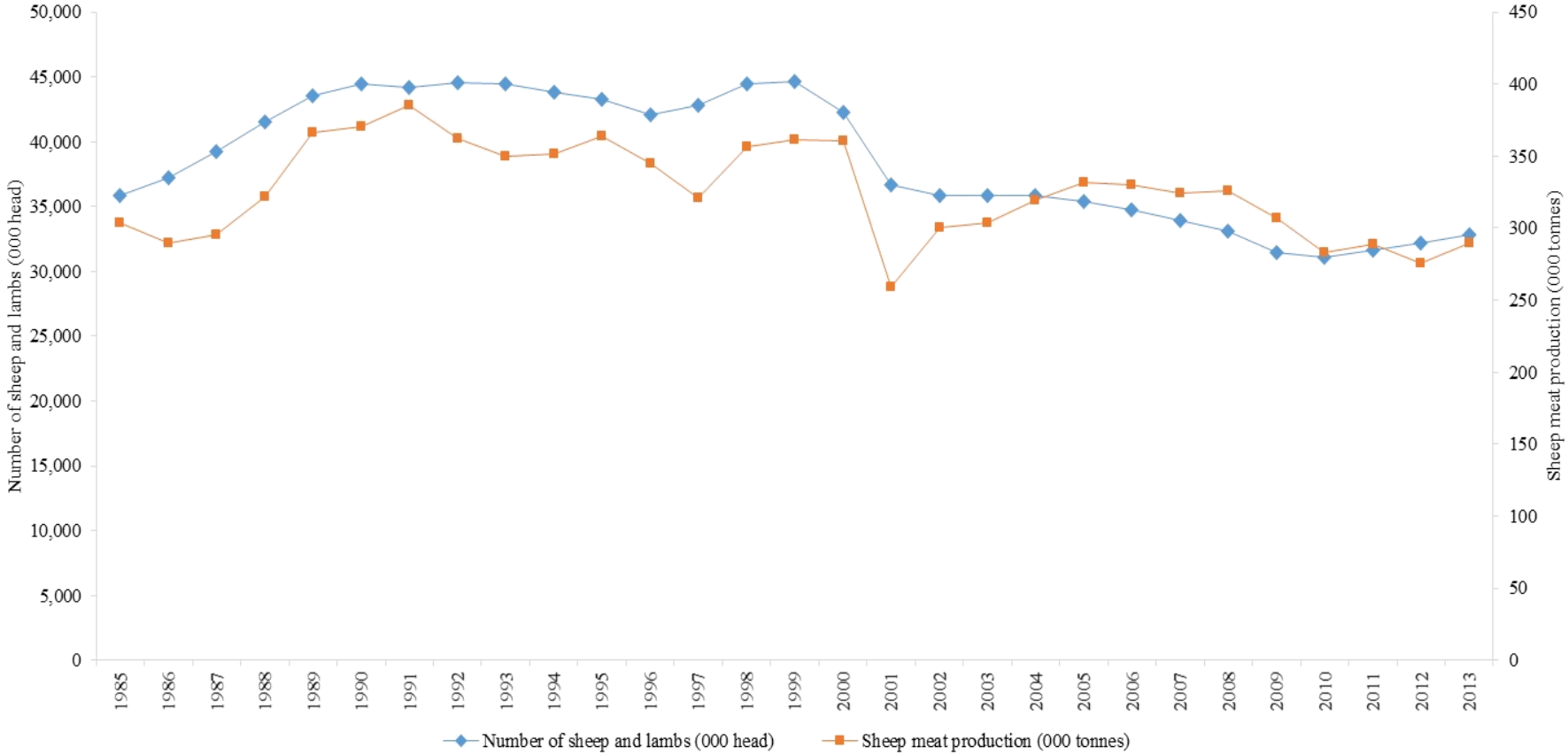


Figure 1.3 Time series data for the number of sheep (ewes intended for breeding and/or slaughter, rams and other sheep one year and over) and lambs (sheep under one year old) recorded (in June for each year) in the UK stock and the volume of sheep meat produced in the UK over the years 1985 to 2013. *Source: GOV.UK, 2014a, 2014b.*

1.1.4 The decline in the United Kingdom's sheep numbers: Interrelated factors

The periodical reform of the Common Agricultural Policy coupled with fluctuations in consumer demand and management inefficiencies of the industry are perhaps the most influential aspects that have led to past and present trends in sheep numbers. These factors, along with the issue of increasing global population growth, are also likely to continue shaping the sheep industry for years into the future.

1.1.4.1 The Common Agricultural Policy

The basis for the Common Agricultural Policy (CAP) stemmed from the 1950s. The main objectives of the policy were to increase food productivity and secure a fair standard of living for agricultural communities while providing consumers with a stable supply of quality food at reasonable prices (European Commission, 2013a). When the CAP was introduced, the incentive to produce was facilitated through a large proportion (over 60%) of the EU budget being allocated to agriculture (European Commission, 2013b). This allowed significant expenditure through financial assistance to develop and restructure farms, provide subsidies to aid the export of surplus products and allow payment of high support prices to farmers.

The EU certainly became very successful in increasing agricultural productivity, securing the market with a high degree of self-sufficiency. However, around the 1980s public concern started to emerge over the sustainability of farming. Mountains of surplus products were a common result of high intensity farm practices. In an attempt to resolve this, large financial contributions had to be paid to subsidise losses in exporting these goods at lower than average prices, consequently having a major impact on international market stability.

To address these and a number of other issues, necessary amendments had to be made to the CAPs objectives in response to the changing social and economic conditions. With regular revision since 1992, change has been implemented through schemes such as agenda 2000, the 2003 reform and 2008 'health check'. High price supports and subsidies have been removed and in 2009 the overall financial input towards agriculture support decreased, taking up as little as 41% of the EU budget; this level of budget share essentially frozen until reform (in 2013) and implementation of newly developed schemes (by 2015) (European Commission, 2013b; GOV.UK, 2014c).

The biggest impact on farming and farm management has perhaps been felt through the de-coupling of payment schemes. Complete removal of price support and maintaining just one

direct income support payment means farmers are required to place more focus on the consumers. This means farmers must adapt by producing in response to customer requirement and demand. A more competitive market is created, however, sectors that have started to experience a decline in product demand i.e. the sheep industry, may struggle to maintain and/or renew stock numbers to remain as a key player and contributor to the meat market and economy.

1.1.4.2 Consumers' perception of lamb: the driver of demand

Current consumption levels of lamb per person in the UK are low (Figure 1.4) as it struggles to compete with options such as pork, beef and poultry. This may simply be due to taste preference, but over recent years lamb has unfairly gained the image of an "old-fashioned" type of meat that is also difficult to cook. Beef, poultry and pork products are regarded as more convenient meat choices as they require less preparation time, are quicker to cook and, most importantly, are cheaper.

For the majority of instances when lamb is purchased it tends to be for special occasions due to cuts being more expensive and the question of value for money. Lamb has also been tagged as a particularly "fatty" and "greasy" type of meat on average in comparison to poultry etc. Not only does this add to the issue of extra preparation time spent trimming undesirable excess visible fat (also regarded as very wasteful), but most importantly it tends to deter consumers from purchasing the product, particularly with the addition of negative and confusing media attention on its consumption and risks to health (Wilson, 1992).

Questioning the dietary value of lamb coupled with uncertainty in food safety e.g. 2001 FMD outbreak, the impact of livestock on the increased greenhouse gas emissions, has led to a change in consumer's attitude and opinions towards red meat, causing individuals to reduce their consumption or cut it out of their diets completely. The effect of this and potentially other factors, such as the CAP headage versus area payments, has been reflected through the steady decline of sheep numbers in the UK flock (Figure 1.3). The priority to the consumer is now centred on value for money, animal welfare, a guarantee in food safety and leaner, healthier cuts of quality sheep meat.

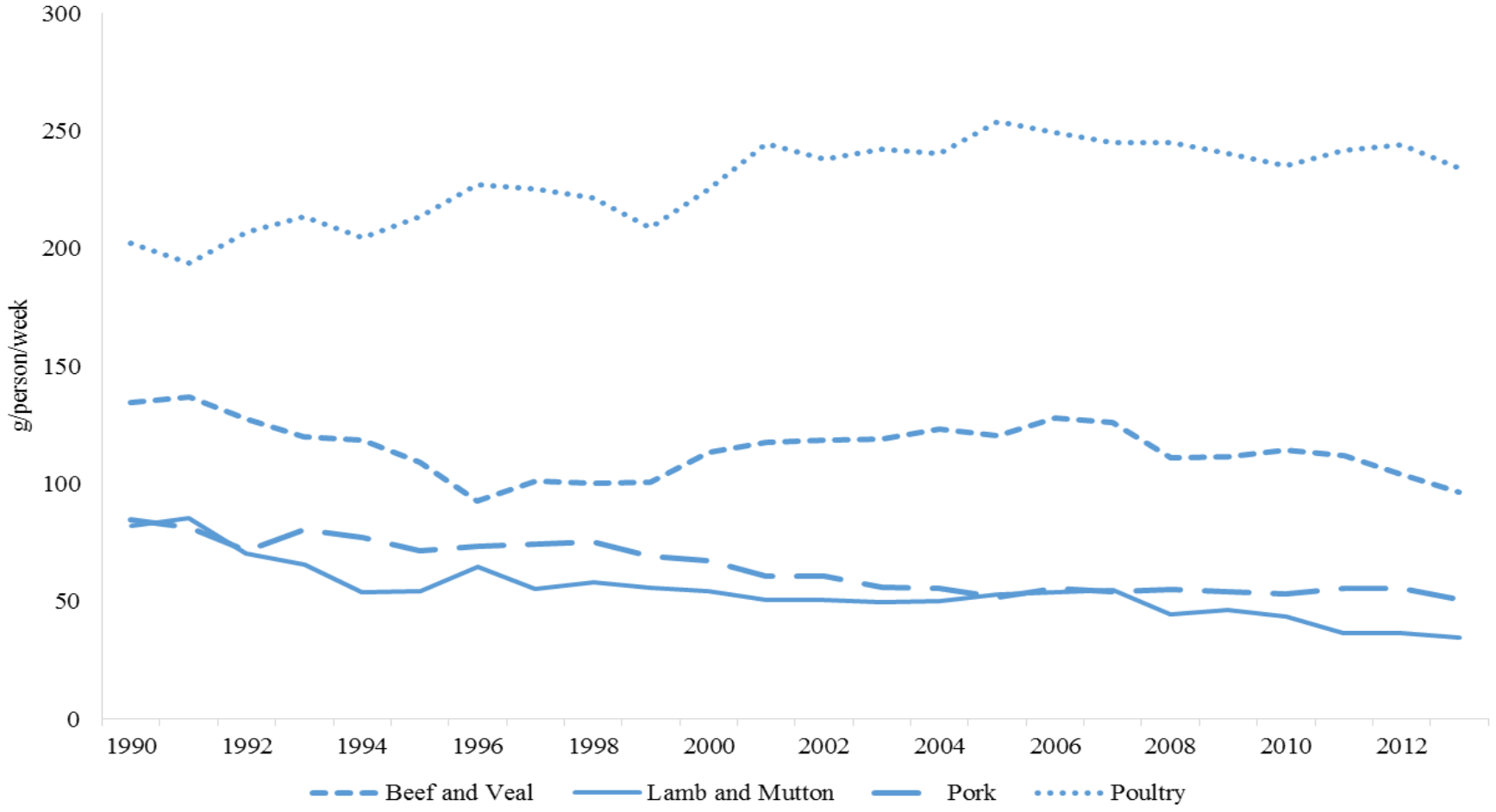


Figure 1.4 Average quantity (g) of main meat items purchased by UK households (per person per week) from 1990 to 2013. *Source GOV.UK, 2014d*.

1.1.5 Measures taken to safeguard the United Kingdom's sheep industry

As previously stated, lamb production is the primary function of the UK sheep industry. In order to maintain current demand and to attract more buyers, the sheep industry must acknowledge consumer requirements. Equally important is the requirement to further improve efficiency of production in the sheep industry. This can be achieved in a number of ways including (but not limited to) addressing net costs of production in terms of profit per ewe e.g. breeding for fertility (improving number of lambs per ewe), breeding for feed efficiency, carcass quality (improving saleable meat yield per lamb), growth etc.

One approach to aid in addressing these challenges is by means of livestock improvement by genetically selecting the breeding animals; stock is improved in terms of the favourable traits selected upon meaning the delivery of the best possible product to market meeting both producer and consumer requirements.

1.2 Livestock improvement

Livestock improvement is not a new concept, it has been ongoing since animals were first domesticated. However, the development and sophistication of technology and genetic methods over recent times have offered new avenues for livestock producers to more effectively manage the efficiency, sustainability and profitability of their production systems to achieve more rapid livestock improvement.

1.2.1 Genetic selection

The environment can be defined as those aspects of farm management that are not accountable by genetics (Warner et al., 2010) i.e. rearing regimes, pre-slaughter conditions, nutrition and feeding/grazing systems (concentrate or pasture). Environmental factors and how they are regulated affect carcass weights and tissue composition and structure throughout growth. For example, the level of important fatty acids and the structure of muscle fibres and connective tissues can be altered, incidentally changing the quality of meat, for example, in terms of tenderness, taste and nutritional value (Popova, 2007; Warner et al., 2010).

While immediate effects can be achieved by altering the management of these environmental aspects, the extent to which they can be modified is limited and has been reported to have comparatively small effects on carcass improvement against that attainable by genetic selection (Lord et al., 1988). Hence, livestock improvement by genetic selection is now the predominant method utilised in enhancing the efficiency of livestock production. The initial

progress is slower but subjective assessment of an individual's performance "by eye" only is removed and, overall, the approach is one that is cumulative, permanent, cost-effective and in line with current emphasis on sustainability (Simm, 1998).

Through the process of genetic selection (or selective breeding) in livestock species, numerous economically important traits are commonly manipulated to achieve a specific breeding goal or set of breeding objectives. The principle of this process is too select a proportion of animals to become parents that will most improve the genetic level for these economically important traits in the next generation (Strandberg and Malmfors, 2006a, 2006b). Analysing the superiority/inferiority of phenotypes for the traits of interest together with pedigree information can provide an estimate of the genetic merit for individual animals.

The basis to identify and select high genetic merit animals firstly includes submitting recordings for all animals for a set of performance traits (e.g. litter size, eight week weight, 21 week weight, ultrasound muscle depth, fat depth etc.). From this point, a statistical procedure BLUP, Best Linear Unbiased Predictor, is used to analyse pedigree and performance data to separate environmental and genetic effects on the phenotype to provide the breeding potential of each animal. The analysis takes into account the performance of the animal itself as well as that of its relatives and ancestors for the performance traits, the relationship between the animals, the known relationship between recorded performance traits (correlations) and the degree to which each trait is inherited from one generation to the next (heritability; h^2) (HCC, 2004; Redden, 2012; AHDB Signet Breeding Services, 2014a, 2014b)

The breeding potential for each trait is assigned to each animal in the form of an Estimated Breeding Value (EBV). The EBVs are presented in the same units as the recorded trait e.g. kg for eight week weight EBV, mm for muscle depth EBV, and expressed relative to a common baseline for all animals in that contemporary group from when recording started (a breed benchmark) (HCC, 2004; EBLEX, 2014c; AHDB Signet Breeding Services, 2014a, 2014b). Ultimately, the EBV for each trait is a prediction of what proportion of superiority (or inferiority) of the animal's genetics, on average, will be passed to progeny if kept and used as breeding stock. The EBV must be halved as each parent contributes half of its genetics to progeny, for example, a ram with a +8kg EBV for eight week weight (8WW) would pass on a +4kg improvement in the average performance of the next generation. If this ram was mated to a ewe with a recorded EBV of +4kg, the average improvement in performance of the next generation would be +6kg. (Figure 1.5).

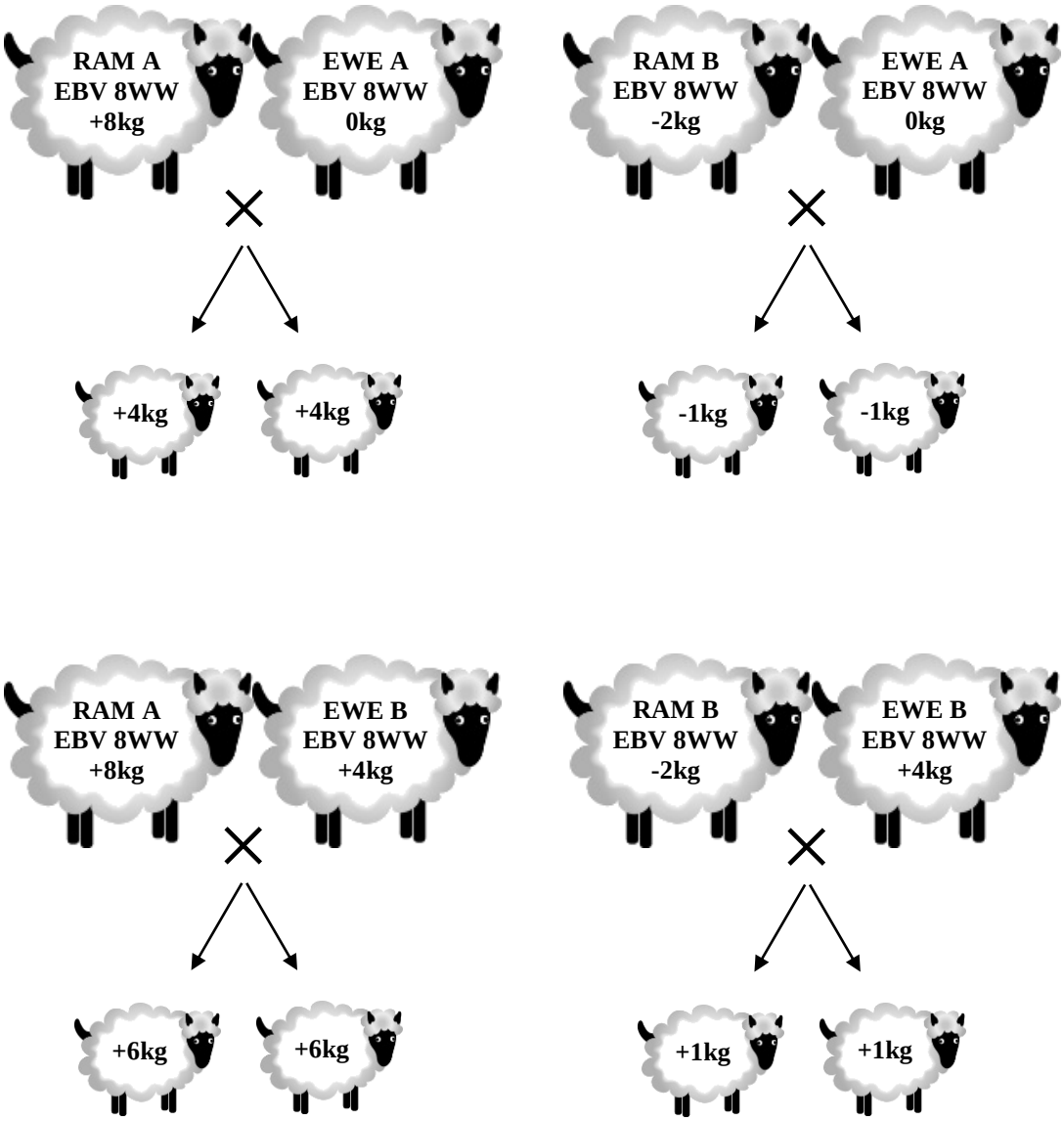


Figure 1.5 Example of the difference in average performance for eight week weight, 8WW, (kg) of lambs produced from rams and ewes with different Estimated Breeding Values (EBVs). Diagram adapted from Redden, 2012.

The collection of EBVs for each trait for each individual animal and their combined use in a selection index is an invaluable tool to accelerate stock performance in accordance with a breeding objective or set of objectives. A selection index (e.g. Terminal Sire Index) combines several EBVs, which are weighted to reflect the traits' emphasis in the breeding goal, into a single index value (Figure 1.6) (AHDB Signet Breeding Services, 2014b). The index value enables the performance for these groups of traits to be quickly and accurately ranked across animals. The higher an animal's index value the higher its overall performance is for the group of traits in that selection index, hence the quicker the set breeding objective will be reached if that animal is used. A selection index is an efficient way to target several traits at once pertaining to the breeding and economic goals of individual producers.

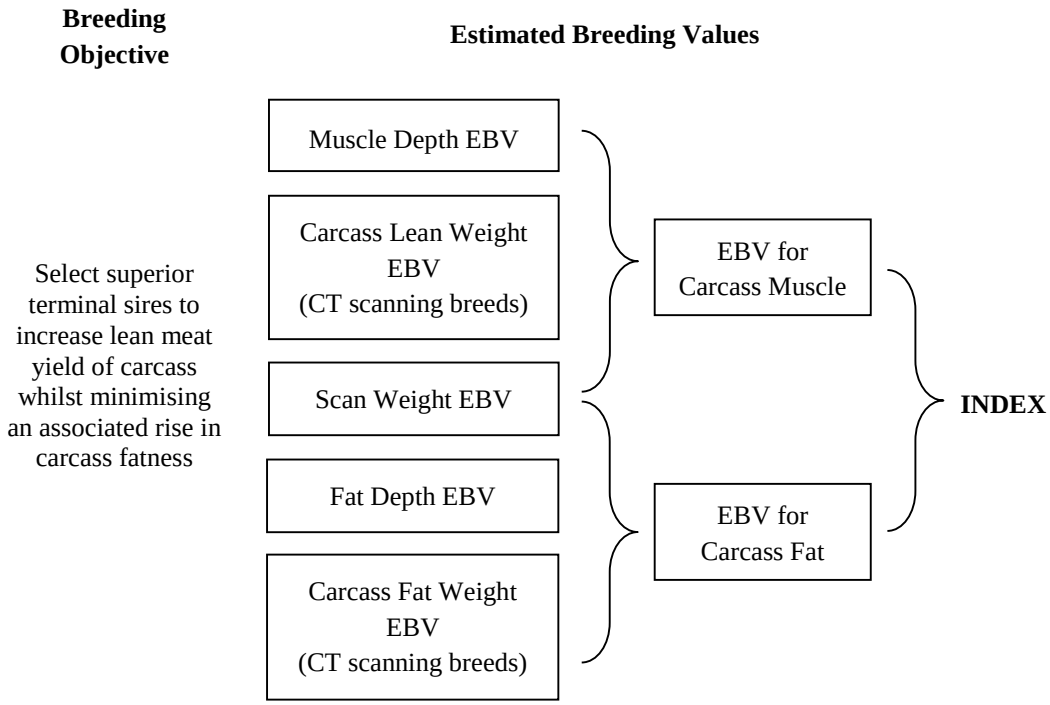


Figure 1.6 Diagram highlighting the breeding objective and the combined Estimated Breeding Values (EBVs) of the traits included in the Terminal Sire Index. Diagram adapted from AHDB Signet Breeding Services, 2014b.

Quality Meat Scotland's Scottish Sheep Strategy group recently investigated the benefits of using high index (top 5% of the breed) performance recorded rams (of the Suffolk breed) with high EBV values for growth traits (eight week weight, scan weight, muscle depth and backfat depth) over rams where the genetic merit was unknown (selected 'by eye') (QMS, 2013a). Sires were mated to randomly selected ewes in three commercial farms over two seasons of lambing (2011 and 2012). Results clearly demonstrated that lambs sired by a high index ram outperformed the lambs that were sired by rams selected 'by eye'. The lambs produced from matings with high index sires were worth an additional annual average of £0.55 to £3.09.

Growth rate is perhaps one of the easier performance traits to measure and continues to be heavily selected upon. However, not all traits are easy to measure and record, but continual advancements in the technology progressively used in agriculture, and increased access to these tools, is now making it much easier to implement genetic selection for the more 'difficult to measure' traits. For example, the traditional method for evaluating the carcass and its genetic merit required the slaughter of the animal. This provides detailed carcass measurements but hinders the progress of overall stock improvement; the slaughtered animal and any desired traits are no longer available for breeding and this evidence is alternatively used as a basis for selection of relatives (Martin and Fredeen, 1966).

Phenotypes expressing desirable carcass traits can now be measured at higher accuracy and more rapidly by the application of partial and full-body scanning methods; ultrasound and X-ray computed tomography (CT) scanning (see section 1.3). These provide reliable *in-vivo* predictions of livestock carcass traits (e.g. fat depth, tissue proportion, distribution and shape), allowing the top percentage of genetically "elite" animals to be selected and used to "breed for a better product" in response to consumer requirements.

1.2.2 A two-stage selection strategy to improve carcass quality of sheep

The current carcass grading system used in the UK and the EU is based on the EUROP classification for conformation and fatness (EBLEX, 2013b). The aim for most farmers and producers is to achieve the optimum in weight and grade (conformation and fatness) which returns profit and meets consumer demand. The majority of slaughter lambs are produced from crosses of different breed-types as a means to meet the optimal carcass weight and grade specification, but a high percentage of these lamb carcasses are still classed as over-fat; in 2012 only 62% produced in the UK met target fat class and conformation grades (QMS,

2013b). It is therefore important that genetic selection for improved carcass quality takes place in the parental breeds (Macfarlane and Simm, 2008).

Terminal sire breeds contribute around 44% of genes to the genetic make-up of slaughter lambs (Pollott and Stone, 2006). Selection for higher quality carcass traits (increased lean tissue growth, muscularity etc.) in these ram stocks is an effective method to consequently improve the carcass quality of the slaughter lambs (Pollott and Stone, 2006; Macfarlane and Simm, 2008). These desirable or "superior" carcass traits that are commercially valuable are identified and measured in the live animal using ultrasound and CT scanning.

Ultrasound is a widely used and effective technique to obtain and assess subcutaneous backfat and muscle depth in sheep (Wilson, 1992; Jones et al., 2002). However, image quality can be poor, measurements imprecise and the technique less informative about the distribution of lean and fat in other areas of the carcass; measurements detail only one section of the animal (Simm, 1987; Simm and Dingwall, 1989; Stanford et al., 1998; Jones et al., 2002). Scanning with CT, on the other hand, offers high prediction accuracy (R^2), over 90%, for total weights of fat, muscle and bone and provides the opportunity to take a much more comprehensive set of measurements which describe the whole carcass (Young et al. 1999; Bunger et al., 2010).

However, due to the higher cost of CT scanning, it is not feasible to scan every animal with this procedure; therefore, implementing a two-stage scheme is beneficial. Firstly, this involves all animals to be scanned in the field using a mobile, less costly application such as ultrasound. This is used as a pre-screening method to identify the top 15 - 20% of candidate ram lambs i.e. those observed to perform highest for chosen traits (e.g. muscle depth), which are then sent to be further CT scanned (Macfarlane and Simm, 2006, 2008).

1.3 Computed tomography: Measuring new, potentially exploitable, skeletal traits

Over the last 20-30 years, means for accurately and reliably measuring body composition have been developed. Traditionally carried out by dissection, taking measures of body composition now generally relies on the use of technologies originally developed for use in human diagnostics and therapeutics; these have been widely reviewed (e.g. Speakman, 2001; Scholz et al., 2015). Computed Tomography (CT) is one such technique and is regularly used in current agricultural practice as it offers a non-invasive image based technique for whole body composition analysis and carcass evaluation *in vivo*.

As, for example, discussed by Bunger et al. (2011) and Scholz et al. (2015), CT scanners use monochromatic X-rays to generate cross-sectional, two-dimensional anatomy images (also called slices or tomograms) of the body. The procedure to generate these images involves transmitting a narrow beam of low dosage X-rays, from an X-ray source, in thin slices through the body of the animal. An X-ray detector(s) is/are aligned with the X-ray source but on the opposite side of the animal and measures the attenuation of the transmitted X-rays (discussed below). The X-ray source and X-ray detector(s) are mounted within the gantry of the scanner and rotate 360° around the body of the animal as it moves through the gantry of the CT scanner on a motorised table at a computer controlled speed. This allows the X-ray beam to pass through the body of the animal from all angles (Young et al., 2001) (Figure 1.7).

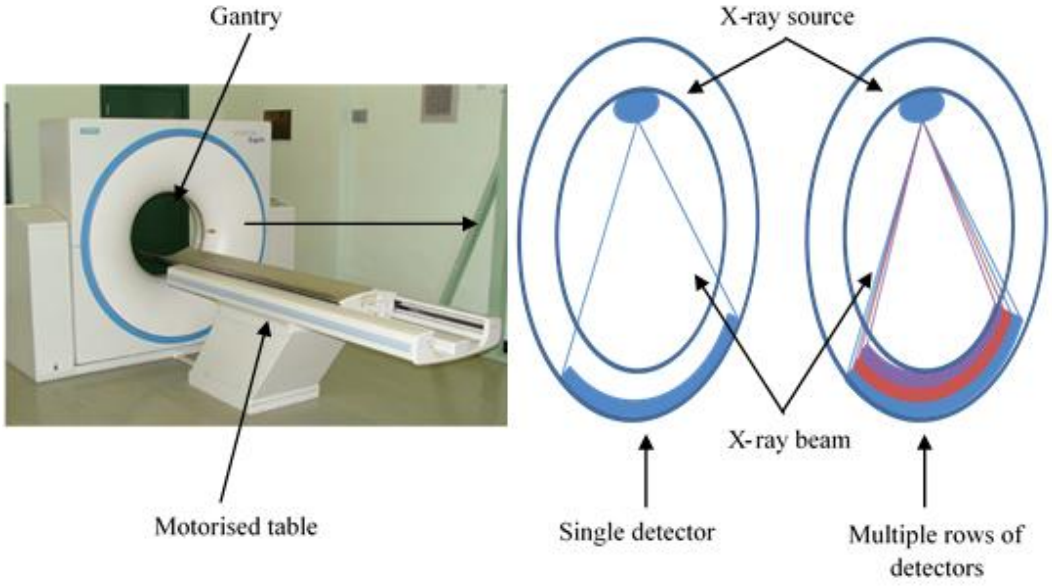


Figure 1.7 Image of a computed tomography (CT) scanner used at Scotland's Rural College (SRUC), Edinburgh. The animal is placed on the motorised table which moves through the gantry of the CT scanner. The gantry holds the X-ray source which transmits a beam of X-rays through the body of the animal. This rotates 360° around the animal as it is moved through the gantry. The attenuation of the X-ray beam is measured by either one detector (single-slice devices) or by multiple rows of detectors (multi-slice devices).

In early (single-slice) devices the motorised table upon which the animal is held moves in a step by step manner through the gantry, imaging a single slice at a time as the table and subject remains static. In this instance, one full rotation of the X-ray source and X-ray detector

constructs one two-dimensional slice of the body. In more modern (multi-slice spiral) CT scanners the table moves at a continuous rate through the gantry in co-ordination with the 360° rotating X-ray source and X-ray detectors. Multi-slice CT scanners have multiple rows of detectors, rather than a single row in the earlier devices (Figure 1.7), and can therefore construct multiple (16 to 64 are common) two-dimensional high resolution slices simultaneously in one full rotation. This means a larger area of anatomy can be imaged at one time making the whole process much quicker and reduces the X-ray exposure to the animals.

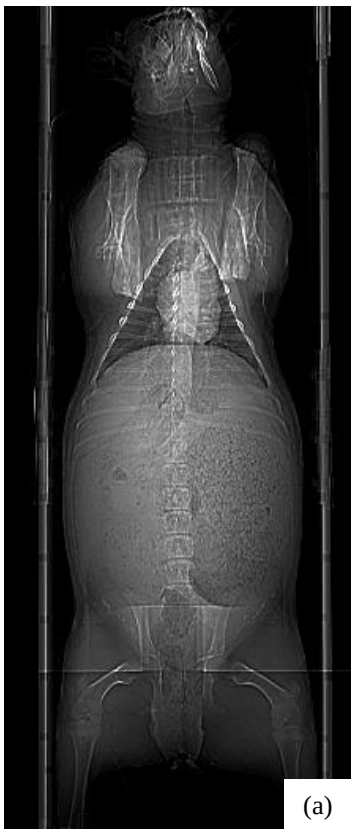
The final two-dimensional cross-sectional images (tomograms) produced (Figure 1.8(b)) are composed of a matrix of tiny squares (pixels, usually $<1mm^2$) which represent 3D blocks of tissue (voxels), which are the thickness of the slice (these can be set to different depths). The colours black and white and shades of grey are assigned to each pixel which relates to the relative density of the tissue in that pixel. The density, and hence type, of tissue is determined by how much of the X-ray beam is absorbed or scattered (attenuated) by the tissue, measured by the X-ray detector(s), as the X-ray passes through the body of the animal (Hathcock and Stickle, 1993). The intensity of the X-ray beam which is attenuated is converted to numerical data and assigned to each pixel in the slice. The attenuation values correspond to a particular colour in the grey scale and produces the final spatial image of the scanned object (Wegener, 1993). The range of values assigned to the various tissues is from +1000 to -1000 (Hounsfield scale); bone = +1000 (bright white), water = 0 (central grey), air = -1000 (black), other tissues are assigned relative to these in grey scale. This provides superior tissue contrast and differentiation within the slices (Hathcock and Stickle, 1993).

Using the CT function of computer aided imaging, these spatially consecutive, high resolution slices can be digitally reconstructed to produce a three-dimensional (3D) representation of the animal. Furthermore, the numerical data comprising the slices and hence 3D images can also be summed and offers the opportunity for estimates of volumes and dimensions of body tissue components in the carcass to be calculated from the images (Krause, 1999). A key aspect of the use and interpretation of CT scanning is the choice and application of software for this type of image analysis. For the purpose of tissue measurement in sheep, special software is needed to extract and quantify the areas of tissues of interest (Glasbey and Young, 2002). The initial action is to segment the image by dividing it into regions or categories of interest, like the carcass portion of *in vivo* scans. This involves applying an algorithm in which each pixel is allocated to a particular category so that pixels in the same category have the same grey scale value (Glasbey and Horgan, 1995). This is then followed by measurement of the areas of

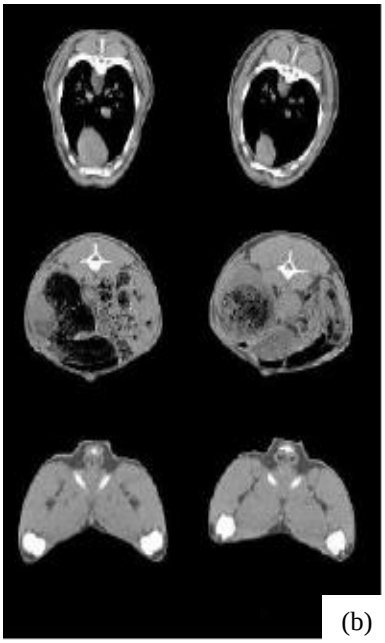
interest in the image. Manual approaches are time consuming and complex, but automation of all these procedures has now been achieved and the challenges and complexities of removing unwanted structures such as internal organs have been addressed and overcome (Glasbey and Young, 2002; Navajas et al., 2006; Mann et al., 2008).

A reference scanning method elaborated in Bunger et al. (2011) has been developed to maximise the accuracy of tissue weight prediction in lambs. Measures from cross-sectional CT reference scans taken at only three specific anatomical locations, the ischium, the 5th lumbar vertebra and the 8th thoracic vertebra (specified as the most informative reference scans to estimate carcass composition), which are identified from a longitudinal topogram scan (Figure 1.8(a)), are combined together with live weights in prediction equations to achieve this. The basis for this was so that highly accurate predictions of carcass composition could be determined from as few scans as possible. This approach has the advantage of not only minimising scanning time, benefiting animal welfare and keeping costs down, but also importantly providing breed specific prediction equations, the basis of the CT scanning of commercial lambs.

Breed specific prediction equations are calibrated against detailed slaughter and dissection information from trial sheep populations of the breeds/crosses concerned and produce very high prediction accuracies, but it is also important to note the limitations which have been considered through the development of the prediction equations. Such as, the calibration populations that were slaughtered and dissected will not be an exact representation of all populations of the breeds/crosses over time; if there is continuous genetic selection then this could change the distribution of tissue across the carcass making the equations less reliable. Using so few reference scans is less feasible when genetic progress is much quicker, such as in pig breeds, but for sheep breeds genetic progress has not moved as quickly. As part of the on-going sheep research work at Scotland's Rural College (SRUC), where there have been trials that include CT scanning then slaughter and dissection, efforts have been made to check that tissue weights predicted with the use of CT and application of prediction equations still match well against the dissection data. To date, this seems to be the case across different breeds and crosses (Nicola Lambe, personal communication).



(a)



(b)

Figure 1.8 Example of output images, topogram (a) and tomograms (b), generated by computed tomography scanning procedure. Different shades of grey represent the different body tissue types: fat (dark grey), muscle (light grey) and bone (white).

The potential for using CT in animal production has been recognised since the 1980s (e.g. Allen and Leymaster, 1985) and the background to its development from that time to current day has been widely reviewed e.g. with a particular focus on its role in the sheep industry by Bunger et al. (2011). In sheep, in general, the procedure has been successfully used to determine aspects such as carcass lean and fat weights, muscularity and body composition with high precision (Lambe et al., 2007). The use of CT has resulted in a much higher genetic gain in sheep stock and a significant increase in production of saleable lean meat yields (Simm and Murphy, 1996; Simm et al., 2002). With the images permitting excellent discrimination between fat, muscle and bone they have the potential to provide a reliable means to also objectively assess variation in skeletal or, more specifically, spine characteristics: spine (or carcass) length, number of vertebrae and length of vertebrae.

1.4 The vertebral column

1.4.1 Spine characteristics and their variation amongst mammals

The spine is part of the endoskeleton and is the backbone in vertebrate animals. Its construction is strong but flexible giving the body support and stability whilst also providing attachment of muscle and protection of the spinal cord. The repeating units that comprise the whole mammalian spinal column are the vertebrae. Starting from the base of the skull, these bones run the length of the dorsal side of all vertebrate animals to the pelvis; the vertebrae series then ends at the coccyx in humans and tailless primates, and at the tip of the tail in the remaining mammalian species.

Distinguishing the morphology of each unit in the spine allows the vertebrae to be grouped into five distinct regions which appear in the fixed consecutive order of cervical (neck), thoracic, lumbar, sacral and caudal (tail). Despite significant changes in many elements of body plans over the course of evolution, the morphologically differentiated groups comprising the spine are a common feature observed across all mammalian groups (Narita and Kuratani, 2005; Wellik, 2007).

It has been well-documented that the vertebrae number in the mammalian cervical region is highly conserved at a total of seven; thought to be in place for at least 200 million years (Buchholtz and Stepien, 2009), mainly as a result of evolutionary constraint against major developmental abnormalities (Galis, 1999). At present, the only three examples recorded to exhibit a departure from this vertebral constant include the three-toed (*Bradypus*) and two-toed (*Choloepus*) sloths and manatees (*Trichechus*) with cervical counts 8-10, 5-8 and 6 respectively (Buchholtz et al., 2007; Buchholtz and Stepien, 2009; Hautier et al., 2010).

The total number of mammalian thoracolumbar (thoracic plus lumbar) vertebrae tends to be 19 (Mikawa et al., 2011). However, in comparison to the cervical region, selection against a change in vertebra number in these more caudal regions is much weaker (Galis et al., 2006) and vertebrae counts for this spinal section frequently vary across mammalian orders. For example, Monotremata, Marsupialia, Lagomorpha, Rodentia and Artiodactyla thoracolumbar vertebrae counts generally show a fixed number of 19, whereas in Perissodactyla (e.g. horse) and Carnivora (e.g. dog) there is an increase, 24 and 20, respectively (Mikawa et al., 2011), however these respective counts fluctuate between species and members of the same species.

These findings raise the question of how these differences in vertebral number may arise. Vertebral number is fixed at some point during development *in utero* hence developmental differences may be an important factor. The literature on the detailed embryonic development in sheep is not large and a lot is still unknown about the precise details of embryonic and foetal development. Nevertheless, overall it would appear that the basic processes are likely to be similar across mammalian species and although not the specific focus of this thesis, a consideration of the development of the spine and associated musculature is relevant here.

1.4.2 Embryonic development of the spine and tissue components

The gestation period in sheep is approximately 145 days, although gestation can vary from 138-159 days. During this period the embryo undergoes a series of transformations as the tissue and organs of the foetus form. The trunk of the body forms quite early in embryogenesis and provides axial symmetry to the developing embryo (Kimmel et al., 1995). Almost all bone and muscle have a mainly embryonic mesodermal origin (Taniguchi et al., 2015), but with each tissue undergoing a cascade of different processes leading to differentiation and growth.

During embryogenesis, the paraxial mesoderm forms bilateral balls of cells known as somites on each side of the neural groove. As development progresses the somites differentiate to form the dermomyotome, which forms muscle, and the sclerotome, which forms the vertebral column. The dermomyotome develops into the myotome and the dermatome (Figure 1.9).

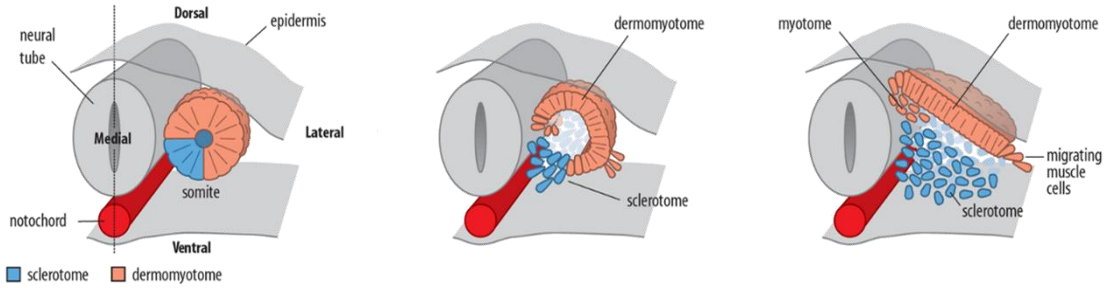


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Figure 1.9 Somites form as balls of cells on either side of the neural tube. The somites differentiate to produce populations of cells; the dermomyotome, which forms muscle and dermis, and the sclerotome, which forms the vertebral column.

The myotome then develops into the muscle of the body and limbs while the dermatome develop into the dermis. The myotome develops further to form a dorsal epimere and a ventral hypomere the cells of which migrate to form the muscles of the trunk. Cells from the epimere

migrate dorsally to form the epaxial, extensor muscles of the vertebral column while cells from the hypomeres migrate to ventral of the vertebral column forming the lateral and ventral muscles of the trunk (Fletcher and Weber, 2015; Pansky, 2015).

The formation of the vertebrae (and ribs) occurs in three main stages. The first is a pre-cartilage stage when somitic segmentation is lost by the migration of cells from the sclerotome to form a continuous mass around the notochord and neural tube. This occurs early in development; in humans during week four. During the second stage, called chondrification, and which occurs at six weeks in humans, centres of chondrification are formed. These fuse at the end of the embryonic period and extensions from the centre in the vertebral arch produce the spinous and transverse processes. The final stage is ossification where there are three centres of primary ossification, one in the centrum and one on either side of the vertebral arch. The final stage starts in the embryonic period and continues into adulthood – around 25 years of age in humans. At birth in humans the three bony parts of each vertebra are connected by cartilage, during the postnatal period the vertebral arch fuses, at puberty five secondary ossification centres are formed and by 25 years of age the secondary centres unite with the rest of the vertebra (Pansky, 2015).

Despite the importance of the sheep as an agricultural animal there is relatively little specific or detailed information on the stage of development at which vertebral number is fixed in sheep. It would seem probable that vertebral number is dependent on the number of somites. However, this is currently an active area of research and it is unclear whether one somite equals one vertebra, hence, the relationship between somites and vertebral number remains speculative. Nonetheless, Nourinezhad et al. (2013) made a macromorphometric study of thoracic vertebra in foetal sheep. The data presented suggest that vertebral number did not change between six and 20 weeks of foetal age, whereas total length of the thoracic vertebral segment increased. Hence it could be speculated that at least the thoracic vertebral number may be fixed by six weeks of gestation.

The mechanisms and molecular control of differentiation and growth of bone and muscle are complex but are widely reviewed elsewhere (Olsen et al., 2000; Buckingham and Rigby, 2014; Schiaffino et al., 2013). However, the development of the vertebral column and muscles of the back and trunk are closely related, not only because of the embryonic origin of the progenitor cells, but also because the tissues share many common factors which control their differentiation and development. These shared regulatory systems may be particularly

important in terms of genetic selection where desired changes in muscularity for example could impinge on bone development such as vertebral number or length. Consequently, the potential to select for one trait independent of another trait may be complicated by molecular processes regulating development.

1.4.3 Why variation in spine characteristics apply in livestock selection

Animal body (and carcass) lengths differ from individual to individual. The diversity in length is immediately associated with and almost completely determined by variation in spine characteristics, specifically, the variation in vertebra number and the individual lengths of these bones in the thoracolumbar spine region (Berge, 1948; King and Roberts, 1960). The *longissimus thoracis et lumborum* (LTL or loin) muscle is highly valuable in the livestock industry as it is a source of quality cuts of meat and important commercially due to its sale at higher prices. The loin muscle runs the length of the thoracolumbar spinal section so in turn is affected by body length (Freeman, 1939; Borchers et al., 2004; Tohara, 1967) i.e. animals with shorter backs (reduced number or length of vertebrae) have a reduced loin length.

In the past, the bacon pig has been extensively selected for an increased body size/length. Favouring the breeding of these larger animals through the years has appeared to have led to the inadvertent selection of individuals that possess an increased number of thoracolumbar vertebrae. Counts of vertebrae in this spine region show a wide variation within one species (Fredeen and Newman, 1962a). The commercial pig breeds are now commonly reported to possess a number of thoracolumbar vertebrae in the range of 21 to 23; a considerable difference to the uniform 19 possessed by its ancestor, the wild boar (*Sus scrofa*) (Mikawa et al., 2007; Yang et al., 2009).

King and Roberts (1960) have reported that that for each additional thoracolumbar vertebra in the pig spine, there was an average increase in length of 15mm. The potential economic benefits that could be gained from the selection of longer animals are indicative from the assessment of meat to fat ratios carried out by Tohara (1967) and Borchers et al. (2004), reporting that animals with a higher number of vertebrae had more meat and less fat. Further to this, thoracolumbar vertebrae numbers have been reported to be highly heritable (Berge, 1948; Fredeen and Newman, 1962b; Borchers et al., 2004; Tohara, 1967).

1.5 Thesis aims and outline

The volume of meat processed from a lamb carcass is variable, even at a standardised live weight or carcass weight. The advent and application of both ultrasound and CT technologies have provided a means to more accurately measure and select for total carcass fat and lean weight as outlined above. However, considerable value could be added to these measurement techniques if traits to predict the distribution of muscle or saleable meat yield for specific body regions or cuts could be included. Hence, the inclusion of thoracolumbar spine characteristics (and consequently loin length/chop number, Figure 1.10) as traits for genetic selection in sheep breeding could prove to be of considerable importance in terms of sheep meat production.

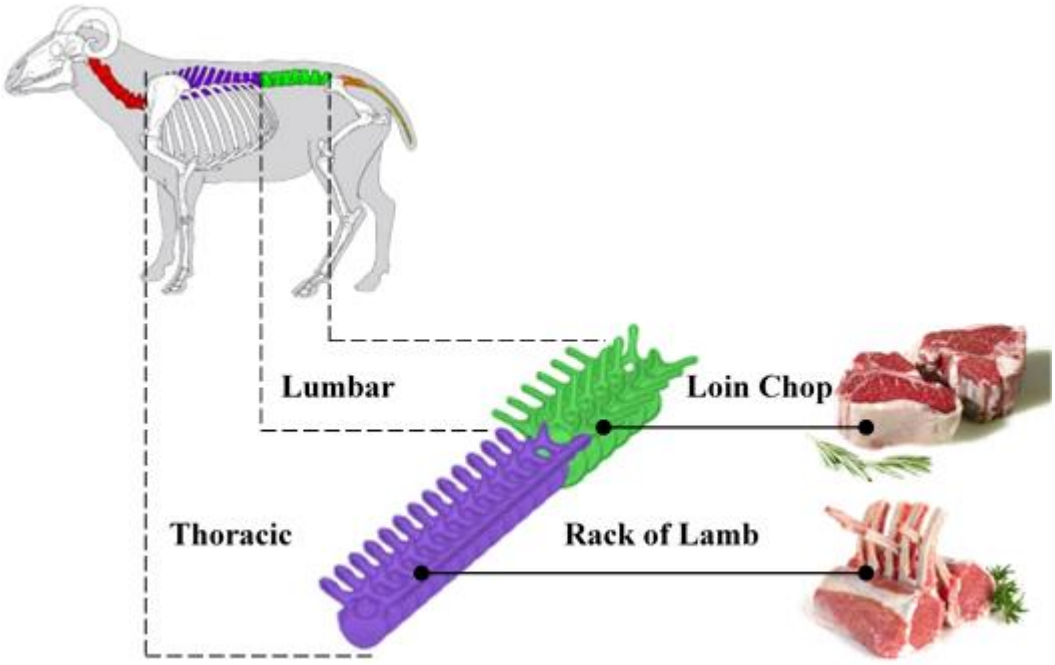

Figure 1.10 Diagram showing the location of thoracic and lumbar regions within the spine and the related sheep meat cuts.

The question was first posed by a leading Scottish sheep breeder and was underpinned by the observations in pig breeds (as described above). The sheep industry questioned whether genetic selection of similar spine traits in sheep could successfully achieve an average increase in chop number and/or size per generation. The aim of this thesis was to explore this possibility by addressing the following questions:

1. Can computed tomography (CT) be accepted as an accurate and reliable method to use in order to quantify spine characteristics (spine length, vertebrae length and vertebrae number) *in vivo*, for the thoracic, lumbar and thoracolumbar spine regions of sheep?
2. What is the degree of variation in these spine trait phenotypes within and between different breeds and crosses of sheep?
3. How do these spine traits correlate with each other and how are they correlated with other economically important production traits?
4. With the loin and (thoracolumbar) spine closely associated, does the effect (localised muscle hypertrophy of the loin) of the Texel muscling quantitative trait locus (TM-QTL) also subsequently affect the underlying spine characteristics?
5. After estimation of genetic parameters for these spine traits and of their genetic correlations with other economically important production traits, what is the initial conclusion to the potential gains from including spine traits in selective breeding programmes?

Answering the above questions was approached through three studies (presented in the thesis as three Chapters). Chapter 2 addresses questions 1 – 3. First, by assessing intra- and inter-operator repeatability of spine measurements from topogram scans, the reproducibility of measures and the reliability of using the CT (scan) measurement method could be assessed. With confirmation of its reliability, spine traits which were recorded (using the CT measurement method) in a selection of sheep breeds/crosses, representative of the divergent genotypes found in different levels of the stratified UK industry, were analysed to describe the extent of variation in spine traits within these breeds/crosses and to determine any significant genotypic effect on these traits. Having established marked differences in spine traits within and across these sheep breeds/crosses, in order to determine if additional vertebrae in the thoracolumbar spine region contribute to an increase in carcass/body length, the phenotypic correlations between the spine traits were estimated and examined. Phenotypic correlations between the spine traits and production traits were also examined in order to investigate how the variation in spine traits may relate to changes in economically important traits.

Question 4 is addressed in Chapter 3 and continues with exploring the potential of utilising the variation in spine traits to improve meat yield. In the case of this study, Texel sheep with increased muscling (through the effects of the TM-QTL and its inheritance) which also possess

a higher number of thoracolumbar vertebrae may prove valuable for further consideration with this study's aim in mind. Initial investigations into the association between spine traits, increased muscling of the loin and the inheritance pattern of the TM-QTL were undertaken in Chapter 3. The final question, concerning the genetic analysis of spine traits, is addressed in Chapter 4. Using estimated variance and covariance components, genetic parameters for the spine traits and genetic correlations between traits were calculated. This information is important to assess so as to supply pragmatic recommendations on introducing the use of spine traits in a commercial breeding situation. Chapter 5, the general discussion and final conclusions of the thesis project, summarises the overall opportunities, implications and areas for future development which were highlighted through conducting the studies.

Chapter 2

Between- and within-breed variations of spine characteristics in sheep

2.1 Introduction

The vertebrate spinal column comprises a series of repeating bones called vertebrae. These bones are variable in size and their morphological differences sub-divide the vertebrae series into five functionally distinct spinal regions: cervical (C), thoracic (T), lumbar (L), sacral (S) and caudal (Cd). Counting the number of vertebrae that comprise each spinal region provides the vertebral formula, e.g. for the majority of humans this is C7 T12 L5 S5 Cd4 (Willis, 1923; Treuting and Dintzis, 2011). In mammals, the cervical component of these formulae rarely show intra- or inter-species variation, remaining at a fixed total of seven for the majority of species (Galis, 1999; Hautier et al., 2010). In contrast, variation is common in the vertebrae combinations of post-cervical regions both between (e.g. Owen, 1853) and within species (e.g. Green, 1939; McLaren and Michie, 1954; Stecher, 1962; Pilbeam, 2004).

The findings regarding vertebrae variation in the thoracolumbar (thoracic plus lumbar) region of the bacon pig is of particular interest to livestock breeders. The commercial selection for breeding stock with longer backs means commercial pigs can possess up to four more vertebrae than the ancestral 19 (Fredeen and Newman, 1962a; Mikawa et al., 2007; Yang et al., 2009; Mikawa et al., 2011). This manner of selection may have the potential to increase meat yield from the commercially valuable *longissimus thoracis et lumborum* (LTL, or loin) muscle which is located along the length of the thoracolumbar spinal section. Hence, obtaining similar knowledge regarding vertebrae variation in sheep could prove to be of considerable importance in terms of meat production. The breeds/crosses of sheep used in this study were a selection of those representative of the divergent genotypes found in the different levels of the stratified three-tier crossbreeding structure currently used in UK sheep production. They included Scottish Blackface (maternal breed stock), Texel (terminal sire breed) and Poll Dorset and Texel crosses (three-way cross slaughter lambs).

The objective of the following study was therefore to use vertebrae/spine measurements and production trait records for the above breeds/crosses to

(i) Summarise the extent of variation in spine traits in the thoracolumbar spine region of sheep and assess if significant differences exist between the sexes and/or breeds/crosses

(ii) Examine, within breed/cross, how spine traits correlate with each other and with selected tissue traits (total predicted fat and muscle in the carcass and area of the loin) related to production

2.2 Materials and methods

2.2.1 Animals and data set

The study for the present chapter was conducted using tissue and spine measures from 1,858 lambs. Records included female and entire male lambs reared as either singles, twins or artificially (pet) from Texel (TEX), Scottish Blackface (SBF), Texel cross Mule (TEX x MULE) or Poll Dorset cross Mule (PD x MULE) breeds/crosses. The rationale for selecting the breeds and crosses used was twofold. Firstly, TEX (as the most numerous terminal sire breed), SBF (as the most numerous hill breed) and Mule crosses (as the most numerous crossbred) are the basis of the sheep industry in Scotland and secondly, TEX are highly selected for lean growth whereas SBF are not, whilst the crossbred lambs contain genes from both. The ewes used for breeding were of mixed age (Table 2.1) (in this study, Mule ewes were Bluefaced Leicester cross SBF).

Table 2.1 Summary of data by breed/cross[1].

Breed/Cross	N	Sex		Rearing rank			Dam age range (years)
		Male	Female	Single	Twin	Pet	
TEX	254	110	144	103	137	14	2 – 6
SBF	1,100	560	540	485	611	4	2 – 7
TEX x MULE	326	154	172	31	295	.	3 – 6
PD x MULE	178	86	92	.	178	.	4 – 5
Total	1,858	910	948	619	1,221	18	

[1] TEX = Texel; SBF = Scottish Blackface; TEX x MULE = Texel cross Mule; PD x MULE = Poll Dorset cross Mule

All lambs had been scanned using X-ray computed tomography (CT); a non-invasive technique that allows a wide range of measurements to be collected from the animal *in vivo* (described in Chapter 1 but with more detailed descriptions of the procedure from Jones et al. (2002) and Bunger et al. (2011)). These scans were taken over the years 2003 to 2008, with the lambs at an average age of 107 days (TEX; range 90 – 119 days), 120 days (SBF; range 95 – 153 days), 132 days (TEX x MULE; range 114 – 152 days) and 113 days (PD x MULE; range 108 – 117 days). Live weight (LWT) of lambs was recorded immediately before they were CT scanned; average weight (kg) for each group was 33.6 (TEX, SE = 0.38), 29.6 (SBF, SE = 0.14), 37.7 (TEX x MULE, SE = 0.26) and 31.1 (PD x MULE, SE = 0.24). All tissue and spine traits (defined in next section) were measured post-CT scan with the use of cross-sectional reference scans and topograms, generated from the CT procedure.

2.2.2 Trait measurements derived from computed tomography

2.2.2.1 Tissue traits

Pixel analysis of cross-sectional CT reference scans allows the area of each different tissue type, fat, muscle and bone, to be derived (see Glasbey and Robinson, 2002). Application of the appropriate breed-specific prediction equations to these values, such as those developed and used by Lambe et al. (2003), provides a reliable prediction of whole body tissue volumes/weights. In this study, prediction of total carcass fat (kg) and muscle (kg) were included (Pfat and Pmusc respectively) along with an estimate of area (mm^2) for the loin (LD_A), as measured from the cross-sectional scan taken at the 5^{th} lumbar vertebra. Including traits Pfat, Pmusc and LD_A in the current study was to provide an initial indication of any possible changes in production traits (i.e. muscularity/lean meat yield) that may be associated with variations in spine traits.

2.2.2.2 Spine traits

The two-dimensional (2D) topograms of each lamb were analysed using Sheep Tomogram Analysis Routines software (STAR, version 4.17), developed jointly by Biomathematics and Statistics Scotland (BioSS) and SRUC. Similar to the cross-sectional reference scans, these longitudinal images of the animal's body permit excellent discrimination between the tissue types, fat, muscle and bone, allowing vertebrae to be counted and lengths of desired spinal regions to be measured. Figure 2.1(a) is an example of a typical topogram and highlights the spine regions of interest: thoracic, lumbar and thoracolumbar (thoracic plus lumbar).

Four of the nine spine traits included in the data set: length (mm) of the thoracic (SPL_{THOR}) and lumbar (SPL_{LUM}) spine regions and number of thoracic (VN_{THOR}) and lumbar (VN_{LUM}) vertebrae, were measured directly from each topogram by one of the four protocol-trained operators involved in the analysis of CT images. The protocol defined for measuring spine characteristics from CT scans closely followed that previously described by Jones et al. (2002), and which has also been used in Navajas et al. (2007).

Firstly, before the measurement procedure is described, it is important to note that vertebrae were classified as thoracic when bearing symmetric or asymmetric ribs: true (attached to sternum) or rudimentary, while vertebrae bearing no ribs and positioned between the cranial side of the pelvis and the most caudal positioned thoracic vertebra were identified as lumbar. The trait SPL_{THOR} was then measured as the distance from the intervertebral disc immediately caudal to the last thoracic vertebra to the intervertebral disc immediately cranial to the first

thoracic vertebra, and SPL_{LUM} was measured as the distance from the intervertebral disc positioned to the cranial side of the pelvis to the first intervertebral disc caudal to the last thoracic vertebra. The number of vertebrae belonging to each of these sections (VN_{THOR} and VN_{LUM}) was then counted. Figure 2.1(b) provides a diagrammatic representation of these measurements.

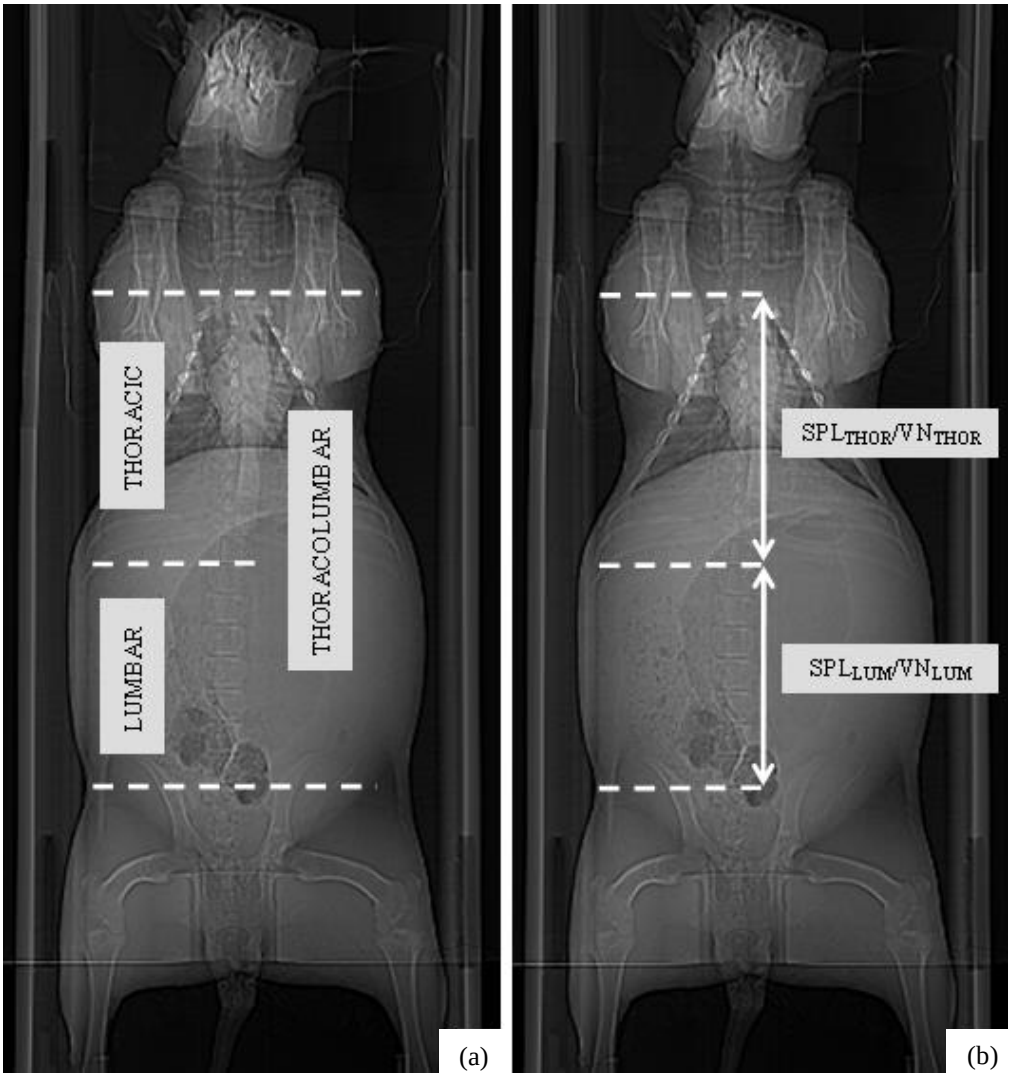

Figure 2.1 Example 2D topogram generated from computed tomography (CT) scanning. Classification of vertebrae allows the boundary (represented as broken white lines) between the cervical-thoracic (top), thoracic-lumbar (middle) and lumbar-sacral (bottom) spinal regions to be identified and the location of the spine regions of interest to be highlighted (a). The intervertebral discs positioned at these boundaries can then be used as reference points for taking length measures (SPL) and vertebral counts (VN) directly from the topogram for the thoracic ($_{THOR}$) and lumbar ($_{LUM}$) spinal regions (b).

The spine traits SPL_{THOR}, SPL_{LUM}, VN_{THOR}, and VN_{LUM} were then used to derive the length (mm) of the thoracolumbar spine region (SPL_{T+L}) and the number of thoracolumbar vertebrae (VN_{T+L}) as follows:

Length of thoracolumbar spine region (SPL_{T+L}) = SPL_{THOR} + SPL_{LUM}

Number of thoracolumbar vertebrae (VN_{T+L}) = VN_{THOR} + VN_{LUM}

Finally, with the use of all of the above measurements, an average length for individual vertebrae (mm) in each spine region could be derived as follows:

Average length of individual thoracic vertebrae (VL_{THOR}) = SPL_{THOR} / VN_{THOR}

Average length of individual lumbar vertebrae (VL_{LUM}) = SPL_{LUM} / VN_{LUM}

Average length of individual thoracolumbar vertebrae (VL_{T+L}) = SPL_{T+L} / VN_{T+L}

2.2.3 Intra- and inter-operator repeatability of spine measurements

An important point to highlight is that the initial classification of vertebrae from topograms requires a subjective decision, i.e. to which region a single vertebra should be allocated. It is therefore important to have a detailed protocol in place, particularly when multiple operators are involved, to reduce, as far as possible, the influence of an individual's judgement on results. The repeatability and agreement of measurements within and between operators, after using a fixed protocol, was evaluated, to validate CT as a reliable method for quantifying spine characteristics.

A total of 100 topograms of TEX (n = 47) and SBF (n = 53) were used for the analysis. Spine traits SPL_{THOR}, SPL_{LUM}, VN_{THOR} and VN_{LUM} were scored directly from the topograms by three operators, coded as A, B and C, following a fixed protocol (as described in previous section). This was carried out twice for each topogram by each operator, the repeat being carried out at least 24 hours after the first run of measurements. Operators A and B did this for the total 100 scans, while C analysed 50 of these scans (TEX, n = 25; SBF, n = 25). The spine traits SPL_{T+L}, VN_{T+L}, VL_{THOR}, VL_{LUM} and VL_{T+L} are not included in this test as the measurements recorded for these traits were simply derived from SPL_{THOR}, SPL_{LUM}, VN_{THOR} and VN_{LUM}.

2.2.4 Statistical analysis

All data were analysed using SAS, version 9.1, (SAS Institute Inc., Cary, NC, USA). To investigate the reliability of the method used to quantify the spine characteristics, ANOVA mixed model analyses for repeated measures were performed to calculate the intraclass correlation coefficient (r_t) to estimate intra- and inter-operator repeatability of spine trait measures taken from CT topograms.

The ANOVA generalised model procedure was used to analyse the effects of breed and sex on spine length (SPL_{THOR}, SPL_{LUM}, SPL_{T+L}, VL_{THOR}, VL_{LUM}, VL_{T+L}) and spine count (VN_{THOR}, VN_{LUM}, VN_{T+L}) traits. Fitted in the model as fixed effects were breed, with four levels (TEX, SBF, TEX x MULE and PD x MULE), sex, with two levels (male and female), dam age, with six levels (2, 3, 4, 5, 6 and 7 years), and rearing rank, with three levels (single, twin or pet). The significance of interaction between fixed effects and each trait were tested and final models altered for the count and length traits separately. With dam age non-significant for all length traits, the fixed effects in the final length trait model included breed, sex and rearing rank. Sex and dam age were shown to be non-significant for count traits, therefore, fixed effects included in the final count trait model were breed and rearing rank. Each of the fixed effects included in the final models were significant for all or the majority of traits.

All of the above models were run once with no covariate adjustment and once with an adjustment for LWT. Doing so, in terms of the biological nature of vertebrae number, should reveal that this meristic characteristic of the spine, once determined genetically in early development (Burke et al., 1995), will not then be influenced by environmental factors (such as nutrition) later in life; results are hypothesised to remain the same for each instance (i.e with no LWT adjustment and with LWT adjustment in model). With regards to spine length traits, it was of interest to investigate if any particular breed/cross exhibited significantly longer spine regions and/or vertebrae (no LWT adjustment in model) and if these differences were removed when comparing the groups all at the same weight (LWT adjustment in model). The least-squares means for each breed and sex and standard errors of difference between the groups were generated for each trait.

Phenotypic correlation coefficients (r_p) were also examined between all CT spine and tissue traits (Pfat, Pmusc, LD_A) to derive any trait associations. Fitted as a covariate in the model, LWT was significant for all spine length traits and tissue traits and non-significant for the majority of spine count traits. Correlations of residuals were therefore estimated, by breed,

after spine length traits were adjusted for sex, rearing rank and LWT, tissue traits adjusted for sex, dam age, rearing rank and LWT and spine count traits adjusted for rearing rank. In this study (and in Chapter 4) the degree of correlation was categorised into six levels (as described in Williams and Monge (2000)); very high ($r \geq 0.90$), high ($0.90 > r \geq 0.70$), moderate ($0.70 > r \geq 0.50$), low ($0.50 > r \geq 0.30$), little, if any ($r < 0.30$) and non-significant ($P > 0.05$).

2.3 Results

2.3.1 Intra- and inter-operator repeatability of spine measurements

Quantifying spine traits from CT topograms can be accepted as a reliable method as high levels of reproducibility for spine measures were observed when recorded either by the same or different individuals following a fixed protocol (Table 2.2). Intra-operator intraclass correlation coefficients varied from high to very high for all spine characteristics (observer A, r_t = 0.82 to 0.93; observer B, r_t = 0.78 to 0.88; observer C, r_t = 0.77 to 0.83). Similarly, inter-operator intraclass correlation coefficients revealed that acceptable levels of agreement were achieved for the majority of operator paired comparisons; only did the agreement for the spine trait VN_{THOR} drop below a moderate correlation level in some cases (Table 2.2).

Table 2.2 Intraclass correlation coefficients (r_t) for intra- and inter-operator repeatability of spine measurements taken from CT topograms.

Trait	Intra-operator			Inter-operator		
	A:A	B:B	C:C	A:B	A:C	B:C
SPL_{THOR}	0.93	0.85	0.82	0.77	0.65	0.67
SPL_{LUM}	0.90	0.88	0.83	0.86	0.85	0.89
VN_{THOR}	0.82	0.78	0.77	0.46	0.46	0.81
VN_{LUM}	0.90	0.86	0.80	0.83	0.82	0.87

CT = x-ray computed tomography

2.3.2 Intra- and inter-breed variation in spine traits

2.3.2.1 Spine count traits

As far as it is known, variation in the spine characteristics of interest to the present project (spine region length, vertebrae length, but in particular vertebrae number) have not been previously explored to any significant extent in general terms, let alone with specific reference to the possibility of their use in selection to alter the carcass, for example in shape and/or size, in order to improve meat production. Therefore, it was important to firstly assess the raw data and report the array of variation in the number of thoracolumbar vertebrae and in the thoracic-lumbar vertebral formula within breeds, a summary of which is provided in Table 2.3.

Table 2.3 Number (and percentage) of lambs within each breed[1] which belong to each thoracolumbar vertebrae number category. Within each combination of breed – thoracolumbar vertebrae number category, lambs were divided further according to their thoracic-lumbar vertebral formula.

Thoracolumbar vertebrae number	Thoracic(T)-Lumbar(L) vertebral formula	TEX (n = 254)		SBF (n = 1,100)		TEX x MULE (n = 326)		PD x MULE (n = 178)	
17	T13 L4	1	(0.39)						
18	T12 L6	5	(1.97)	5	(0.45)	2	(0.61)	3	(1.69)
19	T12 L7	28	(11.0)	20	(1.82)	11	(3.37)	10	(5.62)
	T13 L6	149	(58.7)	372	(33.8)	156	(47.9)	61	(34.3)
20	T12 L8			1	(0.09)				
	T13 L7	62	(24.4)	633	(57.5)	146	(44.8)	95	(53.4)
	T14 L6	8	(3.15)	52	(4.73)	11	(3.37)	8	(4.49)
21	T13 L8			1	(0.09)				
	T14 L7	1	(0.39)	16	(1.45)			1	(0.56)

[1] TEX = Texel; SBF = Scottish Blackface; TEX x MULE = Texel cross Mule; PD x MULE = Poll Dorset cross Mule

Based on the distribution of lamb records (Table 2.3) which were available at the time for the present study, the following are some observations regarding the characteristics of (thoracolumbar) spine count traits specific to each breed,:

(1) The TEX breed exhibited the widest range of thoracolumbar vertebrae number (17 – 21) and TEX x MULE the smallest (18 – 20), while SBF and PD x MULE exhibited an intermediate range (18 – 21).

(2) TEX: despite the larger range of thoracolumbar vertebrae number in TEX, the percentage of animals that possessed the extreme vertebral counts were very low; < 1% of the total sample possessed 17 or 21 thoracolumbar vertebrae (1 lamb in each case). The majority of TEX lambs (~ 70%) actually fell into the 19 thoracolumbar vertebrae category with most of these lambs exhibiting the T13 L6 thoracic-lumbar vertebral group.

(3) SBF and PD x MULE: these two groups were similar in that the majority of the lambs possessed 20 thoracolumbar vertebrae; ~ 62% for SBF and ~ 58% for PD x MULE respectively, of which, most of were recorded to possess the same thoracic-lumbar vertebral formula of T13 L7. Interestingly, the thoracolumbar spine regions

of 17 SBF lambs were comprised of 21 vertebrae. In the other breed/cross groups, if any extreme vertebral counts were observed the number of lambs to which this applied did not exceed one (as observed with this particular data set).

(4) TEX x MULE: the full sample of lambs which belonged to this cross fell almost completely within the 19 and 20 thoracolumbar vertebrae number categories, with a near equal percentage of lambs belonging to each; ~ 51% and ~ 48% fell in the 19 and 20 thoracolumbar vertebrae number categories, respectively. Where the lambs were recorded to possess 19 thoracolumbar vertebrae the most common thoracic-lumbar vertebral formula was T13 L6, and when recorded to possess 20 thoracolumbar vertebrae the most common thoracic-lumbar vertebral formula amongst the lambs was T13 L7.

The above is somewhat reflected in the results from the inter-breed analysis of spine count traits (Table 2.4). From the least-squares means, significant differences could be identified between certain breeds/crosses. In brief, the count traits VN_{LUM} and VN_{T+L} were, on average, significantly lower in the TEX breed compared to the SBF breed and the crosses, whilst VN_{THOR} and VN_{T+L} were, on average, significantly higher in the SBF breed than other groups.

2.3.2.2 Spine length traits

Significant differences were also observed between the breed/cross groups for the length of each spine region ($SPL_{THOR,\ LUM,\ T+L}$) and for the average length of individual vertebrae belonging to each spine region ($VL_{THOR,\ LUM,\ T+L}$) (Table 2.4). For the most part, the crosses were observed to have, on average, longer spine regions and vertebrae in comparison to the TEX and SBF breeds. The lowest values were observed for TEX lambs; however, for some length traits (SPL_{THOR}, VL_{THOR}, and VL_{T+L}) there were no significant differences between TEX and SBF.

2.3.2.3 The statistical model

The breed differences remained consistent for the majority of spine traits across both of the models (no LWT adjustment and with LWT adjustment) giving an indication to a genetic basis for the variation in spine characteristics. To note, sex effects on spine traits were also tested, but for the majority there were no significant differences between males and females; VL_{LUM} was the single trait where significant differences between sexes appeared (males were slightly shorter than females, results not shown).

Table 2.4 Least-squares means (and SE) for CT measured spine traits[1] in different breeds/crosses[2].

Trait	TEX (n = 254)		SBF (n = 1,100)		TEX x MULE (n = 326)		PD x MULE (n = 178)	
SPL_{THOR}	252.7^{c}	(1.719)	254.5^{c}	(1.614)	279.0^{a}	(1.813)	270.8^{b}	(2.043)
SPL_{THOR}_LWT	250.5^{d}	(1.067)	261.7^{c}	(0.999)	265.9^{b}	(1.220)	270.5^{a}	(1.305)
SPL_{LUM}	181.8^{c}	(1.619)	190.9^{b}	(1.521)	199.1^{a}	(1.708)	198.7^{a}	(1.925)
SPL_{LUM}_LWT	181.8^{d}	(1.226)	196.0^{b}	(1.147)	193.4^{c}	(1.401)	200.8^{a}	(1.499)
SPL_{T+L}	434.4^{d}	(2.419)	445.4^{c}	(2.272)	478.2^{a}	(2.551)	469.5^{b}	(2.875)
SPL_{T+L}_LWT	432.3^{c}	(1.397)	457.7^{b}	(1.308)	459.2^{b}	(1.597)	471.3^{a}	(1.708)
VL_{THOR}	19.67^{c}	(0.121)	19.61^{c}	(0.114)	21.59^{a}	(0.128)	20.98^{b}	(0.144)
VL_{THOR}_LWT	19.52^{d}	(0.073)	20.15^{c}	(0.068)	20.63^{b}	(0.083)	20.96^{a}	(0.089)
VL_{LUM}	28.63^{d}	(0.142)	28.84^{c}	(0.133)	30.71^{a}	(0.149)	30.21^{b}	(0.168)
VL_{LUM}_LWT	28.50^{d}	(0.087)	29.49^{c}	(0.081)	29.66^{b}	(0.099)	30.28^{a}	(0.106)
VL_{T+L}	22.63^{c}	(0.114)	22.72^{c}	(0.107)	24.63^{a}	(0.120)	24.09^{b}	(0.135)
VL_{T+L}_LWT	22.50^{d}	(0.061)	23.31^{c}	(0.057)	23.66^{b}	(0.070)	24.12^{a}	(0.075)
VN_{THOR}	12.84^{c}	(0.028)	12.96^{a}	(0.026)	12.92^{b}	(0.031)	$12.90^{b,c}$	(0.035)
VN_{THOR}_LWT	12.84^{b}	(0.028)	12.98^{a}	(0.027)	12.89^{b}	(0.032)	12.90^{b}	(0.035)
VN_{LUM}	6.392^{c}	(0.045)	6.662^{a}	(0.042)	6.527^{b}	(0.049)	6.639^{a}	(0.056)
VN_{LUM}_LWT	6.394^{c}	(0.046)	6.659^{a}	(0.043)	6.534^{b}	(0.052)	6.639^{a}	(0.056)
VN_{T+L}	19.24^{c}	(0.048)	19.63^{a}	(0.044)	19.44^{b}	(0.052)	19.54^{b}	(0.058)
VN_{T+L}_LWT	19.23^{d}	(0.048)	19.64^{a}	(0.045)	19.42^{c}	(0.054)	19.54^{b}	(0.058)

Within a row, means with common superscripts (a-d) are not significantly different ($P > 0.05$)
LWT = live weight (kg) fitted as a covariate in model

[1] CT = x-ray computed tomography; SPL_{THOR} = length of thoracic spine region (mm); SPL_{LUM} = length of lumbar spine region (mm); SPL_{T+L} = length of thoracolumbar spine region (mm); VL_{THOR} = average length of individual thoracic vertebrae (mm); VL_{LUM} = average length of individual lumbar vertebrae; VL_{T+L} = average length of individual thoracolumbar vertebrae (mm); VN_{THOR} = number of thoracic vertebrae; VN_{LUM} = number of lumbar vertebrae; VN_{T+L} = number of thoracolumbar vertebrae

[2] TEX = Texel; SBF = Scottish Blackface; TEX x MULE = Texel cross Mule; PD x MULE = Poll Dorset cross Mule

2.3.3 Intra-breed trait correlations

Further to the investigation of intra-breed vertebral variation, phenotypic correlation coefficients (r_p) among all CT spine and tissue traits were examined; these are given in Table 2.5 for breeds TEX and SBF and Table 2.6 for crosses TEX x MULE and PD x MULE.

2.3.3.1 Spine traits

How variation in spine count traits (VN_{THOR}, VN_{LUM}, VN_{T+L}) were associated with spine length traits (SPL_{THOR}, SPL_{LUM}, SPL_{T+L}, VL_{THOR}, VL_{LUM}, VL_{T+L}) within each breed/cross was assessed. Firstly, correlations between traits concerning the combined thoracic and lumbar (thoracolumbar) spine region showed significant ($P < 0.001$) moderate positive linear associations between VN_{T+L} and SPL_{T+L} (TEX, $r_p = 0.41$; SBF, $r_p = 0.60$; TEX x MULE, $r_p = 0.50$; PD x MULE, $r_p = 0.64$) and between SPL_{T+L} and VL_{T+L} (TEX, $r_p = 0.62$; SBF, $r_p = 0.65$; TEX x MULE, $r_p = 0.64$; PD x MULE, $r_p = 0.50$), but negative correlations between VN_{T+L}

and VL_{T+L} (TEX, r_p = −0.46; SBF, r_p = −0.21; TEX x MULE, r_p = −0.34; PD x MULE, r_p = −0.33). These correlations give an indication that an increased thoracolumbar spine region length may arise from two situations; a higher number of shorter vertebrae or a lower number of vertebrae that are longer.

Assessing the thoracic spine region alone, in TEX, there were significant ($P < 0.001$) positive correlations between VN_{THOR} and SPL_{THOR} (r_p = 0.43) and between SPL_{THOR} and VL_{THOR} (r_p = 0.79), and again a significant ($P < 0.001$) negative correlation between VN_{THOR} and VL_{THOR} (r_p = −0.21). SBF, TEX x MULE and PD x MULE exhibited similar positive correlations for the former two traits, but unlike TEX there were no significant ($P > 0.05$) associations between VN_{THOR} and VL_{THOR}. The higher correlations occurred between SPL_{THOR} and VL_{THOR} within each group (SBF, r_p = 0.85; TEX, r_p = 0.79; TEX x MULE, r_p = 0.88; PD x MULE, r_p = 0.76) which could suggest that if a lamb has a long thoracic region a higher proportion of this is due to individual vertebrae being longer, rather than an increased number of vertebrae.

Investigating associations between spine count and length traits concerning only the lumbar region revealed that all breed/cross groups displayed very strong and significant positive correlations between traits VN_{LUM} and SPL_{LUM} (TEX, r_p = 0.92; SBF, r_p = 0.90; TEX x MULE, r_p = 0.89; PD x MULE, r_p = 0.93) and negative correlations between VN_{LUM} and VL_{LUM} (TEX, r_p = −0.46; SBF, r_p = −0.44; TEX x MULE, r_p = −0.37; PD x MULE, r_p = −0.44). However, non-significant correlations ($P > 0.05$) occurred between SPL_{LUM} and VL_{LUM} for TEX, SBF and TEX x MULE. This may suggest that for these breed/cross groups, if an increase in the lumbar region occurs, a higher proportion of this is due to additional lumbar vertebrae, in contrast to the thoracic where it is more likely to be due to an increase in the length of the individual vertebrae.

2.3.3.2 Tissue traits

Correlations between tissue and spine traits appeared to be more breed/cross specific. The tissue traits Pmusc and LD_A in TEX were only showing low but significant correlations with VN_{THOR}, VN_{LUM}, SPL_{THOR} and SPL_{LUM}; these were positive with VN_{THOR} and SPL_{THOR} but negative with VN_{LUM} and SPL_{LUM}. Hence, TEX lambs that have a longer thoracic and shorter lumbar region may be, on average, more likely to have slightly more muscle in their carcass and a larger loin area, at a given live weight.

For SBF lambs there were no significant correlations between Pmusc and spine traits. On the other hand, low but significant negative relationships were found between Pfat and SPL_{LUM}, SPL_{T+L}, VL_{LUM} and VL_{T+L} and between LD_A and VN_{LUM}, VN_{T+L}, SPL_{LUM} and SPL_{T+L}. This suggests that SBF lambs that were observed to possess a longer lumbar length may also be observed to have a slightly decreased volume of carcass fat and a smaller loin area, at a given weight.

Very few correlations between tissue and spine traits showed significance within the TEX x MULE group; Pfat showed a negative correlation with SPL_{THOR} ($r_p = -0.14$) and LD_A showed a negative correlation with VL_{LUM} ($r_p = -0.11$). Within the PD x MULE lamb group, significant negative correlations occurred between Pmusc and SPL_{LUM}, SPL_{T+L}, VL_{LUM} and VL_{T+L} and between LD_A and VL_{LUM}.

Table 2.5 Phenotypic correlation coefficients (r_p) between CT measured traits[1] for Texel (above diagonal; n = 254[2]) and Scottish Blackface (below diagonal; n = 1,100[3]) lambs.

Trait	Pfat	Pmusc	LD_A	VN_{THOR}	VN_{LUM}	VN_{T+L}	SPL_{THOR}	SPL_{LUM}	SPL_{T+L}	VL_{THOR}	VL_{LUM}	VL_{T+L}
Pfat		**-0.31*****	-0.03	-0.03	0.02	-0.01	-0.05	-0.01	-0.05	-0.03	-0.06	-0.04
Pmusc	0.01		**0.66*****	**0.14***	**-0.16***	-0.05	**0.15***	**-0.20****	-0.06	0.06	-0.06	-0.01
LD_A	0.27***	0.59***		**0.18****	**-0.15***	-0.01	**0.14***	**-0.18****	-0.04	0.03	-0.03	-0.03
VN_{THOR}	0.01	-0.01	-0.03		**-0.36*****	**0.41*****	**0.43*****	**-0.40*****	0.01	**-0.21*****	-0.01	**-0.35*****
VN_{LUM}	-0.05	-0.04	**-0.10*****	**-0.20*****		**0.70*****	**-0.52*****	**0.92*****	**0.41*****	**-0.31*****	**-0.46*****	**-0.19****
VN_{T+L}	-0.04	-0.05	**-0.11*****	**0.37*****	**0.84*****		**-0.18****	**0.60*****	**0.41*****	**-0.47*****	**-0.46*****	**-0.46*****
SPL_{THOR}	-0.05	0.02	-0.01	**0.48*****	**-0.32*****	-0.03		**-0.45*****	**0.50*****	**0.79*****	**0.33*****	**0.64*****
SPL_{LUM}	**-0.08****	-0.04	**-0.14*****	**-0.21*****	**0.90*****	**0.74*****	**-0.20*****		**0.55*****	**-0.21****	-0.11	0.03
SPL_{T+L}	**-0.11*****	-0.02	**-0.13*****	**0.17*****	**0.53*****	**0.60*****	**0.56*****	**0.70*****		**0.54*****	**0.20****	**0.62*****
VL_{THOR}	-0.06	0.03	0.00	-0.04	**-0.24*****	**-0.25*****	**0.85*****	**-0.10*****	**0.54*****		**0.37*****	**0.93*****
VL_{LUM}	**-0.06***	0.02	-0.06	0.04	**-0.44*****	**-0.40*****	**0.32*****	-0.01	**0.23*****	**0.34*****		**0.59*****
VL_{T+L}	**-0.09****	0.02	-0.05	**-0.13*****	**-0.15*****	**-0.21*****	**0.72*****	**0.15*****	**0.65*****	**0.90*****	**0.65*****	

Significant phenotypic correlations in bold; *P < 0.05; **P < 0.01; ***P < 0.001

[1] CT = x-ray computed tomography; Pfat = predicted carcass fat weight (kg); Pmusc = predicted muscle weight (kg); LD_A = area of *longissimus thoracis et lumborum* (mm^2); SPL_{THOR} = length of thoracic spine region (mm); SPL_{LUM} = length of lumbar spine region (mm); SPL_{T+L} = length of thoracolumbar spine region (mm); VL_{THOR} = average length of individual thoracic vertebrae (mm); VL_{LUM} = average length of individual lumbar vertebrae; VL_{T+L} = average length of individual thoracolumbar vertebrae (mm); VN_{THOR} = number of thoracic vertebrae; VN_{LUM} = number of lumbar vertebrae; VN_{T+L} = number of thoracolumbar vertebrae

[2] n = 246 for those correlations against the trait Pfat

[3] n = 1099 for those correlations against the trait Pfat

Table 2.6 Phenotypic correlation coefficients (r_p) between CT measured traits[1] for Texel cross Mule (above diagonal; n = 326) and Poll Dorset cross Mule (below diagonal; n = 178) lambs.

Trait	Pfat	Pmusc	LD_A	VN_{THOR}	VN_{LUM}	VN_{T+L}	SPL_{THOR}	SPL_{LUM}	SPL_{T+L}	VL_{THOR}	VL_{LUM}	VL_{T+L}
Pfat		**-0.27*****	0.11	-0.09	0.06	0.01	**-0.14***	0.06	-0.05	-0.10	-0.01	-0.07
Pmusc	**-0.44*****		**0.53*****	0.07	-0.09	-0.05	0.05	-0.09	-0.04	0.01	0.01	-0.01
LD_A	**0.16***	**0.33*****		0.10	0.03	0.08	-0.01	-0.02	-0.03	-0.07	**-0.11***	-0.10
VN_{THOR}	0.06	-0.03	0.06		**-0.23*****	**0.31*****	**0.43*****	**-0.31*****	0.06	-0.05	**-0.13***	**-0.21*****
VN_{LUM}	0.02	-0.10	0.08	**-0.22****		**0.85*****	**-0.42*****	**0.89*****	**0.47*****	**-0.35*****	**-0.37*****	**-0.23*****
VN_{T+L}	0.06	-0.11	0.11	**0.46*****	**0.77*****		**-0.19*****	**0.70*****	**0.50*****	**-0.37*****	**-0.44*****	**-0.34*****
SPL_{THOR}	-0.04	-0.09	-0.01	**0.59*****	**-0.47*****	-0.04		**-0.33*****	**0.50*****	**0.88*****	**0.30*****	**0.71*****
SPL_{LUM}	0.00	**-0.17***	0.01	**-0.22****	**0.93*****	**0.70*****	**-0.37*****		**0.65*****	**-0.20*****	0.06	0.08
SPL_{T+L}	-0.04	**-0.24****	0.00	**0.27*****	**0.51*****	**0.64*****	**0.46*****	**0.65*****		**0.52*****	**0.29*****	**0.64*****
VL_{THOR}	-0.10	-0.09	-0.06	-0.07	**-0.40*****	**-0.41*****	**0.76*****	**-0.28*****	**0.35*****		**0.40*****	**0.90*****
VL_{LUM}	-0.06	**-0.16***	**-0.21****	0.07	**-0.44*****	**-0.35*****	**0.40*****	-0.11	**0.22****	**0.43*****		**0.71*****
VL_{T+L}	-0.11	**-0.17***	-0.13	**-0.18***	**-0.23****	**-0.33*****	**0.61*****	0.00	**0.50*****	**0.90*****	**0.68*****	

Significant phenotypic correlations in bold; *P < 0.05; **P < 0.01; ***P < 0.001

[1] CT = x-ray computed tomography; Pfat = predicted carcass fat weight (kg); Pmusc = predicted muscle weight (kg); LD_A = area of *longissimus thoracis et lumborum* (mm^2); SPL_{THOR} = length of thoracic spine region (mm); SPL_{LUM} = length of lumbar spine region (mm); SPL_{T+L} = length of thoracolumbar spine region (mm); VL_{THOR} = average length of individual thoracic vertebrae (mm); VL_{LUM} = average length of individual lumbar vertebrae; VL_{T+L} = average length of individual thoracolumbar vertebrae (mm); VN_{THOR} = number of thoracic vertebrae; VN_{LUM} = number of lumbar vertebrae; VN_{T+L} = number of thoracolumbar vertebrae

2.4 Discussion

2.4.1 Deriving spine traits from computed tomography

Early methods used by the livestock sector to measure variation in the thoracolumbar region of commercial pigs included slaughter of the animal and radiography (Martin and Fredeen, 1966). Computed tomography, however, can operate as a more reliable and advanced alternative for measuring spine traits *in vivo*. A computer-aided 3D representation of the animal can be produced from the procedure, providing a means to gain a more comprehensive set of measurements describing the whole carcass. Robust predictions of empirical carcass length can be obtained from the topograms rather than relying on subjective visual judgement.

There is a stratified system of sheep breeding in the UK with the majority of slaughter lambs (~ 70%) sired by rams of terminal sire breeds e.g. Texel, Suffolk, Charollais (Pollott and Stone, 2006). The selection goals in these terminal sire breeds are centred on the breed's size, carcass characteristics and particularly a high lean growth (Bunger et al., 2011), as such, these terminal breeds are used in the final stage of the breeding system in order to produce the 'right' slaughter lamb for market (Pollott and Stone, 2006). Due to its more advanced capabilities, many more rams, normally candidates for selection, are routinely placed under the CT scanning procedure so as to assess their carcass quality, determined by factors such as the composition, proportion and distribution of tissue (e.g. muscle). Therefore, topograms are readily available for spine measurements to be taken. The additional information for spine traits could inform breeders about other areas of potential stock improvement i.e. the prospect of added value from each slaughter lamb if an increase of average carcass length (or length of a high-priced region such as the loin) was achieved.

2.4.2 Learning from pigs

In fact, with the implementation of spine traits in pig selection, increases of up to 15mm in thoracolumbar length have been reported with each additional vertebra present in this region (King and Roberts, 1960). It is this variation in vertebrae number, specifically in the thoracolumbar region, that is a major contributor to the diversity observed in body (and carcass) length (Berge, 1948), i.e. animals have shorter backs if there is a reduced number of vertebrae and *vice versa*. Furthermore, a number of beneficial responses in production traits have been associated with an increased number of presacral vertebrae (combined number of cervical, thoracic and lumbar vertebrae), for example, a decrease in loin fat depth (Borchers et al., 2004). Such benefits have been achieved, and can be maintained, through the genetic

change of spine characteristics; carcass length has been described as a highly heritable trait in pigs (Berge, 1948; Fredeen and Newman, 1962b; Borchers et al., 2004) and QTLs identified on *Sus scrofa* chromosomes one and seven are likely to be associated with an increase in vertebrae number in pigs (Mikawa et al., 2005; Mikawa et al., 2007; Mikawa et al., 2011).

2.4.3 Spine traits in sheep

For the majority of mammals a departure from a total of seven cervical vertebrae is uncommon, on the other hand, variation in the number of vertebrae comprising the post-cervical regions occurs frequently (Galis et al., 2006). The sheep spine is commonly used as a model for investigating musculoskeletal conditions relating to the human spine. A few of these anatomy texts give reference to the pre-sacral vertebral formulae in sheep (Wilke et al., 1997; Lori et al., 2005), highlighting some degree of variability in vertebrae number, but only to their specific study breeds. Unlike the bacon pig, little has been reported on the extent of vertebral variation in sheep with particular reference as to how its investigation may impact the agricultural industry i.e. meat yields, change in shape and composition of carcasses.

This study has aimed to help fill this gap in knowledge. It included the assessment of a large sample of topograms of males and females belonging to four different breeds/crosses widely used in the Scottish sheep industry. The use of TEX and SBF is of particular importance when considering the effect of selective breeding for lean growth on spine characteristics. The TEX are more highly selected in this respect than SBF which tends to be less 'improved'. While little to no significant variation was observed in spine traits between the sexes, the study has revealed marked differences in vertebrae number and length between the breeds/crosses, which are also indicative of a genetic basis to the variation.

Given the relationship between muscle and bone development, it is perhaps not surprising that animals selected for lean growth may show different vertebral characteristics when compared to less intensively selected animals. The average vertebral number and length for TEX was significantly lower than that for SBF, whereas, Mule crosses have intermediate values in comparison, suggesting a relationship between lean muscle and vertebrae number/length. With further consideration to the distribution of VN_{T+L} (based on the thoracic-lumbar vertebral formula groups; Table 2.3), in all breeds/crosses it appears that outliers e.g. TEX with 17 or 21 VN_{T+L}, are common and consistent with a normal type distribution as might be expected with any biological system. However, it may also be possible that some measurement error

due to distortions in the image from spine curvature (see Chapter 5) may have contributed to the results.

The biological basis of the differences reported in this study cannot be fully determined from the present results. However, in terms of development it might be speculated that phenotypic selection for lean muscle growth might alter the balance between myogenic and osteogenic cells or the timing of cell division/migration. Such speculation would be consistent with the observation that the control of vertebral number, which is complex and appears to occur early in development, is achieved through the action of several genes, some of which also appear to play a role in musculoskeletal patterning (Imura et al., 2009; Pineault and Wellik, 2014).

Similarly to pigs, the present study also showed that there was an association between an increased thoracolumbar length and the possession of a greater number of vertebrae. Rather than an instance of a greater number of smaller sized vertebrae with no subsequent increase in overall carcass length, correlations between the traits VN_{T+L} and SPL_{T+L} for each breed/cross support that more vertebrae, albeit slightly shorter (as suggested by the correlations between traits SPV_{T+L} and VL_{T+L}), will still contribute to the animal having a longer thoracolumbar region.

However, having a greater number of vertebrae is not the only source of additional length in the body. Another way by which animals may have a longer thoracolumbar region (revealed by correlations between traits SPL_{T+L} and VL_{T+L}) is through the possession of a smaller number of vertebrae that are individually larger in size. Growth of bones in both instances will to some degree be determined by the availability of a favourable environment. Those animals with the propensity to possess extra vertebrae through genetic inheritance, nevertheless, could have the potential to display improved performance in phenotype for body length over those that express the primitive 19 (or less) thoracolumbar vertebrae.

2.4.4 How changes in spine traits associate with production traits

In terms of changes in the production traits, Pfat, Pmusc and LD_A, there were very few significant directional relationships with the spine traits for each breed/cross and in those that did occur, the magnitude of the correlations were small (r_p = 0.06 – 0.24).

Where Pfat did show a correlation with spine traits, the quantity of fat in the carcass showed a decrease with a longer thoracolumbar region; a result similarly interpreted from comparisons

made in meat to fat ratios between larger- and smaller-bodied pigs by Tohara (1967). This result would be favoured in the current market due to the demand from the consumer for leaner sheep meat (Ward et al., 1995). However, excluding a few incidences in TEX lambs, negative correlations were observed between the production trait Pmusc and spine traits, indicating a decline in the percentage of muscling in the carcass when there is an increase in spine length. The results for Pfat and Pmusc were not consistent over all spine traits however, and for the majority of trait correlations in each breed/cross they were shown to be non-significant. Furthermore, Pfat and Pmusc were traits concerning the carcass as a whole; factors other than a change in spine characteristics, e.g. environment or management, may have a significant influence on such aspects.

LD_A was the only production trait included in this study that concerned the loin area exclusively. Its correlations with the spine traits are very variable across the breeds/crosses. The only situation where positive associations were observed was in the TEX breed; there was an increased LD_A with spine traits VN_{THOR} and SPL_{THOR}. However, considering that, also in TEX, the association between LD_A and traits VN_{LUM} and SPL_{LUM} are negative and non-significant with the thoracic plus lumbar spine traits (VN_{T+L} and SPL_{T+L}), an increase in either the thoracic or lumbar region may be counterbalanced with a reduction in the other, resulting in a non-significant net effect between LD_A and vertebral increase.

2.5 Conclusion

The sheep industry is important in UK exports of lamb meat, but there is still a high requirement for the industry to increase its efficiency further. Regarding pigs, literature has reported associated benefits between bacon production and certain spine traits. Hence, it may be possible that application of spine trait records in the selection of sheep will aid in improving carcass quality in terms of, for example, size and meat yield. This could be a particularly useful method in breeds where there are no associated negative effects on welfare, the breed's spine traits appear lower than average or if there appears to be a higher tendency to possess the primitive 19 thoracolumbar vertebrae, in TEX for instance.

Chapter 3

Effect of the Texel muscling QTL (TM-QTL) on spine characteristics in purebred Texel lambs

3.1 Introduction

Walling et al. (2004) first reported evidence of a quantitative trait locus (QTL) segregating in the United Kingdom's Texel sheep population which significantly increased *longissimus thoracis et lumborum* (LTL or loin) muscle depth (up to +1.15 – +2.00 mm, as measured ultrasonically over the third lumbar vertebra). Observing similar results (QTL effect of +2.57 mm) from an analysis including existing and new Texel family data, the QTL, later termed the Texel muscling QTL (TM-QTL), was further verified by Matika et al. (2006). Located on the distal end of the ovine chromosome 18 (OAR18) (Walling et al., 2004; Matika et al., 2006), the TM-QTL sits in the same region as the *Callipyge* (*CLPG*) and Carwell loci (Cockett et al., 1994; Nicoll et al., 1998) which are also known to affect carcass muscling; the *CLPG* mutation leads to greater muscle mass most pronounced in the hind quarters (loin, pelvis, leg) (Cockett et al., 1994; Koohmaraie et al., 1995; Jackson et al., 1997a, 1997b; Freking et al., 2002), while carriers of Carwell exhibit a larger loin muscle area and weight (Nicoll et al., 1998).

Such QTL are of economic interest as there is the potential to utilise their effects through selection programmes to gain greater carcass value (e.g. reducing fat deposition and increasing lean meat production). In the case of the TM-QTL, the proportion of the high value loin cut may be increased e.g. two-dimensional measurements (estimated from cross-sectional computed tomography (CT) scans, taken at the fifth lumbar vertebra) describing loin depth, width and area were found to be ~ 0.5 – 11% greater in TM carrier lambs than non-carrier lambs (Macfarlane et al., 2010).

Moreover, taking the QTL's mode of inheritance into consideration allows the opportunity to exploit the TM-QTL more fully and appropriately in a commercial situation. Similar to *CLPG* (Cockett et al., 1994; Freking et al., 1998a), expression of the Texel muscling phenotype has been suggested to follow the complex parent-of-origin-dependent pattern of inheritance termed polar overdominance (Macfarlane et al., 2010; Matika et al., 2011). This unique type of inheritance is characterised by the instance where heterozygous progeny that inherit a single copy of the allele from the sire exhibit the superior phenotype (Cockett et al., 1996). Indeed, Macfarlane et al. (2010) observed that the largest phenotypic effects of the TM-QTL were particularly apparent in the TM carrier lambs that had inherited a single copy of the TM allele from the sire and the wild type (+) from the dam (genotype $TM^{S}/+^{D}$; where superscripts S (sire) and D (dam) denote the paternal and maternal origin of the alleles, respectively), with loin depth, width and area measures ~ 2 – 11% greater in these $TM^{S}/+^{D}$ genotype lambs than in the

other three genotype groups (homozygote non-carriers, $+^S/+^D$; heterozygote carriers inheriting TM-QTL from the dam, $+^S/TM^D$; homozygote carriers, TM^S/TM^D).

Essentially, muscle hypertrophy from TM allele segregation appears to be localised to the loin muscle (Macfarlane et al., 2010), which is located along the length of the thoracolumbar (thoracic plus lumbar) spine region. With the development of muscle and bone being closely linked (see section 1.4.2) and the results from Chapter 2, which showed some Texel lambs with an increased length of a specific spine region also possessing a larger loin muscle area (at a given weight), it was of further interest to investigate, across genotype groups, if the change in loin shape/increased loin muscling is associated with any change to characteristics of the underlying spine section i.e. is there a subsequent effect on spine characteristics in relation to the pattern of TM allele inheritance? Freking et al. (1998b), for instance, found that the spinal column was significantly shorter in *CLPG* genotype lambs (-2.5 cm; when all animals compared at the same carcass weight) and the carcasses more compact in skeletal structure in comparison to normal genotype lambs. Given its chromosomal position, it may be a similar condition for the TM-QTL. This is a particularly relevant point to assess in terms of a possible 'trade-off' between increasing loin muscle size (e.g. depth) but, in consequence, shortening the spinal column.

The thoracolumbar spine region encompasses the 'body' (or trunk) vertebrae and the total length of this region (as with any spine region) is a product of the number and length of vertebrae which comprise it. Hence, the difference in body (and carcass) lengths observed from individual to individual is contributed to the variation in these vertebral factors. Recent work, as described in Chapter 2, has demonstrated that the spine characteristics (vertebrae number, vertebrae length), of the thoracolumbar region, can be reliably measured from CT scans. Using this method, it was also identified that these characteristics exhibit significant intra-breed variation in Texel sheep, for example, thoracolumbar vertebrae number was observed to range from 17 to 21. Therefore, it may be reasonable to use CT measured spine and loin traits to investigate if any association exists between the pattern of TM allele inheritance and spine characteristics.

3.2 Materials and methods

3.2.1 Data set

The present study used a subset of the 209 available purebred Texel lamb records previously used by Macfarlane et al. (2010) and Lambe et al. (2011). Lambs were sired by seven different rams that were previously identified as carriers of at least one copy of TM-QTL; all 209 lambs were blood-sampled soon after birth (born 2009) in order to classify their TM-QTL genotype (homozygote non-carrier, $+^S/+^D$; heterozygote carrier inheriting TM-QTL from the sire, $TM^S/+^D$; heterozygote carrier inheriting TM-QTL from the dam, $+^S/TM^D$; homozygote carrier, TM^S/TM^D); detailed information on the genotyping of the animals can be found in Macfarlane et al. (2010). However, for a number of animals the genotype could not be fully classified. These unknowns were excluded from this study's analysis, leaving a total of 142 lamb records in the subset, which divided into the TM-QTL genotype groups as follows: 39 $+^S/+^D$, 52 $TM^S/+^D$, 17 $+^S/TM^D$, 34 TM^S/TM^D. These 142 lamb records included 59 entire males and 83 female lambs from the purebred population of Texel sheep kept across two sites, one in Scotland and one in Wales, and had been reared as either singles (n = 97), twins (n = 34) or artificially (pet; n = 11) (further details on the management of these animals can be found in Macfarlane et al. (2010) and Lambe et al. (2011)).

3.2.2 Trait measurements derived from computed tomography

Lambs were CT scanned at ~ 126 days of age (ranging from 93 to 145 days) and their topogram images (produced from the CT process) used to quantify spine characteristics for each, following the procedure described in Chapter 2. In short, spine traits measured directly from the scans included counts of vertebrae in the thoracic and lumbar regions (VN_{THOR} and VN_{LUM} respectively) and length (mm) of the thoracic and lumbar spine region (SPL_{THOR} and SPL_{LUM} respectively). These measures were used to calculate the average length (mm) of individual vertebrae in the thoracic and lumbar regions (VL_{THOR} (SPL_{THOR}/VN_{THOR}) and VL_{LUM} (SPL_{LUM}/VN_{LUM}) respectively). The results for the thoracic and lumbar spine regions were further used to provide the number of thoracolumbar vertebrae (VN_{T+L} ($VN_{THOR}+VN_{LUM}$)), and the length (mm) of the thoracolumbar region (SPL_{T+L} ($SPL_{THOR}+SPL_{LUM}$)). These thoracolumbar spine traits were then used to calculate the average length (mm) of individual vertebrae across the thoracolumbar region (VL_{T+L} (SPL_{T+L}/VN_{T+L})).

For each lamb, the dimensions, width (mm), depth (mm) and area (mm^2), of the loin (LD_W, LD_D and LD_A respectively), were estimated (from cross-sectional CT scans taken at the

fifth lumbar vertebra) by Macfarlane et al. (2010) and included in this study's analysis of the genotypic effect. Essentially, these traits were included to (i) determine if analysis of the reduced sample of animals shows genotype effects on loin traits similar to that observed for the larger sample and (ii) assess, from further analysis of the smaller data set, if the same or similar pattern of TM expression (polar overdominance) can be considered as a source for any genotype differences.

3.2.3 Statistical analysis

Data were analysed using the GLM procedure in SAS, version 9.1, (SAS Institute Inc., Cary, NC, USA) to determine the effects of genotype on the collated loin traits and measured spine traits. Fixed effects fitted in the model for loin traits (LD_W, LD_D, LD_A), spine length traits (SPL_{THOR}, SPL_{LUM}, SPL_{T+L}, VL_{THOR}, VL_{LUM}, VL_{T+L}) and spine count traits (VN_{THOR}, VN_{LUM}, VN_{T+L}) were site, with two levels (Scotland and Wales), sex, with two levels (male and female), rearing rank, with three levels (single, twin or pet), and TM-QTL-genotype, with four levels ($+^S/+^D$, $TM^S/+^D$, $+^S/TM^D$ and TM^S/TM^D). The model was run with and without covariate adjustments for live weight (LWT); where any of the traits differed significantly between genotype groups, it was of interest to assess if, by testing the groups at a standard live weight, the differences were removed.

A set of orthogonal contrasts, as described by Freking et al. (1998a), (additive (-1 0 0 1), dominance (-1 1 1 -1) and reciprocal heterozygote (0 1 -1 0)) was fitted to the $+^S/+^D$, $TM^S/+^D$, $+^S/TM^D$, TM^S/TM^D genotypes, respectively. The contrasts test for any distinct pattern in the differences amongst the genotype group's least-squares means (for loin and spine traits), from which, a particular model for TM gene action might be suggested. Due to the previous evidence supporting the expression of the TM muscling phenotype through a polar overdominant mode of inheritance (Macfarlane et al., 2010), if significant differences were found between the heterozygote groups (reciprocal heterozygote test) a further set of orthogonal contrasts was fitted to the genotypes to include a test for the paternally derived polar overdominant mode of inheritance. Again following that from Freking (1998a), this second set of orthogonal contrasts included additive (-1 0 0 1), maternal dominance (-1 0 2 -1) and polar overdominance (-1 3 -1 -1) models of gene action which were fitted to the $+^S/+^D$, $TM^S/+^D$, $+^S/TM^D$, TM^S/TM^D genotypes, respectively. Contrasts were performed on the spine count data which was not adjusted for LWT and on the loin and spine length data after the adjustment for LWT.

3.3 Results

In the context of this work it is useful to note that an earlier study (Macfarlane et al., 2012) found that least-squares means for LWT (measured at birth, 5, 10, 15 and 20 weeks of age) and carcass weight for TM^S/TM^D animals were consistently larger than that measured for $+^S/+^D$, $+^S/TM^D$ and $TM^S/+^D$ genotype lambs (these differences were significant between TM^S/TM^D and $+^S/+^D$ lambs for LWT at birth, 5 and 10 weeks of age and carcass weight). In the present study, statistical models were first run without an adjustment of LWT but, given the above, in order to remove, as far as possible, any misinterpretation of TM-QTL effects, statistical models were run again with certain traits (loin dimensions, spine length) adjusted for LWT. The following sections discussing these traits will therefore focus only on the LWT adjusted results.

3.3.1 Loin traits

Similar to the findings of Macfarlane et al. (2010), $TM^S/+^D$ genotype lambs were observed to generally have the largest loin width, depth and area, on average (Table 3.1). The differences in loin dimensions between $TM^S/+^D$ and $+^S/+^D$ genotype lambs were consistently significant, however, the larger trait averages observed for the $TM^S/+^D$ group were not all significantly different from those averages observed for the $+^S/TM^D$ and TM^S/TM^D genotype groups. For example, the $TM^S/+^D$ group was significantly different from the $+^S/TM^D$ group in regards to loin area (LD_A_LWT) but the groups were not significantly different for loin width and depth measures (LD_W_LWT and LD_D_LWT, respectively). Further to this, and in contrast to Macfarlane et al. (2010), the $TM^S/+^D$ group in this smaller data set was not significantly different from TM^S/TM^D with regards to all three loin traits.

In general, the pattern of results from the analysis of the full data set (Macfarlane et al., 2010) suggested that the effect of the TM allele on these loin dimensions is expressed through a non-additive mode of inheritance (paternal polar overdominance). Analysis of the subset of records suggests a more general paternal TM-QTL effect on the loin with little evidence of a polar overdominance effect.

3.3.2 Spine length traits

Overall, there was no significant effect of the TM-QTL on the thoracic region length traits (SPL_{THOR}, VL_{THOR}). Nor was there an effect of TM-QTL genotype on the average length of individual lumbar vertebrae (VL_{LUM}), however, associations were shown to exist between TM-

QTL genotype groups and length of the lumber region (SPL_{LUM}) (Table 3.2). Least-squares means showed that, on average, $+^S/+^D$ and $TM^S/+^D$ genotype lambs had a longer lumbar length compared to $+^S/TM^D$ and TM^S/TM^D genotype lambs. However, when considering the combined length of the thoracic and lumbar regions (SPL_{T+L}), the genotype effect is negligible (Table 3.2).

3.3.3 Spine count traits

The segmentation and anatomical regionalisation of the spinal elements (vertebrae) is established in early development (Wellik, 2007; Iimura et al., 2009), hence, it should not be affected by varying LWT. In running the statistical model with the inclusion and omission of a covariate adjustment of LWT, little difference was found between the least-squares means for each model (Table 3.2), lending support to the previous statement. Therefore, only the results obtained from the model with no LWT adjustment will be discussed (results from the model with LWT covariate adjustment are not shown).

Regarding vertebrae number in the separate thoracic and lumbar spine regions first (VN_{THOR} and VN_{LUM} respectively), there were some significant differences between the genotype groups, however, the magnitude of these differences was relatively small (Table 3.2). In more detail, it can be seen from the least-squares means that there is much overlap between the genotype classes with regards to VN_{THOR}. The $+^S/TM^D$ and TM^S/TM^D genotype lambs had, on average, a greater number of thoracic vertebrae than $+^S/+^D$ and $TM^S/+^D$ genotype lambs, however $+^S/TM^D$ and $+^S/+^D$ genotype lambs were not significantly different from each other. With regards to VN_{LUM}, the $+^S/+^D$ and $TM^S/+^D$ genotype lambs were significantly different from the $+^S/TM^D$ and TM^S/TM^D genotype lambs. While observed to possess fewer thoracic vertebrae, $+^S/+^D$ and $TM^S/+^D$ genotype lambs had a greater number of lumbar vertebrae, on average.

Although significant differences occurred between the genotype groups for the two spine regions when considered separately, when examining the results for the combined thoracic and lumbar vertebrae number (VN_{T+L}), there were no significant differences between the groups (Table 3.2).

Table 3.1 Least-squares means (and SE) for live weight and loin traits[1] for Texel lambs of each TM-QTL genotype[2].

Trait	Genotype								Site	Sex	Rearing rank	TM-QTL genotype	Live weight	R^2
	$+^S/+^D$ n = 39*		$TM^S/+^D$ n = 52*		$+^S/TM^D$ n = 17		TM^S/TM^D n = 34							
Live Weight	30.53	(1.004)	31.78	(0.855)	30.59	(1.402)	31.45	(1.094)	< .001	0.104	< .001	0.672	.	0.253
LD_W	66.27^{b}	(1.034)	69.49^{a}	(0.881)	$67.12^{a,b}$	(1.444)	68.82^{a}	(1.127)	0.084	0.069	0.199	0.034	.	0.126
LD_D	28.45^{b}	(0.779)	30.96^{a}	(0.663)	$29.28^{a,b}$	(1.087)	30.44^{a}	(0.848)	0.234	0.411	0.001	0.029	.	0.163
LD_A	1684^{c}	(64.94)	1883^{a}	(55.33)	$1689^{b,c}$	(90.68)	$1851^{a,b}$	(70.76)	0.385	0.633	< .001	0.021	.	0.157
LD_W_LWT	66.27^{b}	(0.591)	68.43^{a}	(0.507)	$67.06^{a,b}$	(0.825)	68.04^{a}	(0.646)	< .001	0.400	0.059	0.001	< .001	0.716
LD_D_LWT	28.45^{b}	(0.560)	30.28^{a}	(0.481)	$29.25^{a,b}$	(0.782)	29.94^{a}	(0.612)	0.007	0.008	0.148	0.029	< .001	0.570
LD_A_LWT	1684^{c}	(37.68)	1817^{a}	(32.36)	$1685^{b,c}$	(52.63)	$1803^{a,b}$	(41.17)	< .001	0.002	0.353	0.004	< .001	0.718

Within a row, means with common superscripts (a – c), are not significantly different ($P > 0.05$)

LWT = live weight (kg) fitted as a covariate in model

[1] LD_W = width of *longissimus thoracis et lumborum* (mm); LD_D = depth of *longissimus thoracis et lumborum* (mm); LD_A = area of *longissimus thoracis et lumborum* (mm^2)

[2] $+^S/+^D$ = homozygote non-carrier; $TM^S/+^D$ = heterozygote carrier inheriting TM-QTL from sire; $+^S/TM^D$ = heterozygote carrier inheriting TM-QTL from dam; TM^S/TM^D = homozygote carrier

Table 3.2 Least-squares means (and SE) for spine traits[1] for Texel lambs of each TM-QTL genotype[2].

Trait	Genotype								Site	Sex	Rearing rank	TM-QTL genotype	Live weight	R^2
	$+^S/+^D$ n = 39*		$TM^S/+^D$ n = 52*		$+^S/TM^D$ n = 17		TM^S/TM^D n = 34							
SPL_{THOR}	255.6	(3.941)	260.7	(3.358)	257.9	(5.504)	262.5	(4.295)	0.003	0.429	0.034	0.503	.	0.122
SPL_{LUM}	$184.4^{a,b}$	(2.411)	186.8^{a}	(2.054)	176.6^{c}	(3.366)	$180.3^{b,c}$	(2.627)	0.208	0.742	0.029	0.018	.	0.125
SPL_{T+L}	440.0	(5.138)	447.5	(4.378)	434.5	(7.176)	442.8	(5.599)	0.004	0.447	0.015	0.329	.	0.128
VL_{THOR}	20.13	(0.276)	20.60	(0.235)	20.00	(0.386)	20.38	(0.301)	0.009	0.246	0.007	0.352	.	0.133
VL_{LUM}	29.03	(0.292)	29.29	(0.249)	28.91	(0.408)	29.36	(0.319)	< .001	0.702	0.045	0.657	.	0.124
VL_{T+L}	23.10	(0.265)	23.51	(0.226)	22.86	(0.370)	23.28	(0.289)	0.004	0.443	0.007	0.332	.	0.135
SPL_{THOR}_LWT	255.6	(2.495)	256.9	(2.143)	257.7	(3.485)	259.7	(2.726)	0.379	0.456	0.088	0.610	< .001	0.650
SPL_{LUM}_LWT	184.4^{a}	(2.033)	185.2^{a}	(1.746)	176.5^{b}	(2.839)	179.1^{b}	(2.221)	0.151	0.513	0.333	0.007	< .001	0.382
SPL_{T+L}_LWT	440.0	(2.720)	442.0	(2.336)	434.2	(3.798)	438.8	(2.971)	0.061	0.241	0.575	0.289	< .001	0.758
VL_{THOR}_LWT	20.13	(0.160)	20.32	(0.138)	19.99	(0.224)	20.18	(0.175)	0.051	0.772	0.595	0.531	< .001	0.710
VL_{LUM}_LWT	29.03	(0.147)	28.97	(0.126)	28.89	(0.205)	29.13	(0.160)	0.350	< .001	0.011	0.721	< .001	0.781
VL_{T+L}_LWT	23.10	(0.130)	23.22	(0.112)	22.85	(0.182)	23.07	(0.142)	0.023	0.185	0.488	0.297	< .001	0.793
VN_{THOR}	$12.69^{b,c}$	(0.064)	12.65^{c}	(0.055)	$12.89^{a,b}$	(0.090)	12.88^{a}	(0.070)	0.026	0.350	0.044	0.006	.	0.174
VN_{LUM}	6.356^{a}	(0.074)	6.387^{a}	(0.063)	6.111^{b}	(0.104)	6.143^{b}	(0.081)	0.092	0.427	0.061	0.009	.	0.149
VN_{T+L}	19.05	(0.063)	19.04	(0.054)	19.00	(0.088)	19.02	(0.069)	0.759	0.981	0.645	0.967	.	0.011

Within a row, means with common superscripts (a – c), or without superscripts, are not significantly different ($P > 0.05$)

LWT = live weight (kg) fitted as a covariate in model

[1] SPL_{THOR} = length of thoracic spine region (mm); SPL_{LUM} = length of lumbar spine region (mm); SPL_{T+L} = length of thoracolumbar spine region (mm); VL_{THOR} = average length of individual thoracic vertebrae (mm); VL_{LUM} = average length of individual lumbar vertebrae (mm); VL_{T+L} = average length of individual thoracolumbar vertebrae (mm); VN_{THOR} = number of thoracic vertebrae; VN_{LUM} = number of lumbar vertebrae; VN_{T+L} = number of thoracolumbar vertebrae

[2] $+^S/+^D$ = homozygote non-carrier; $TM^S/+^D$ = heterozygote carrier inheriting TM-QTL from sire; $+^S/TM^D$ = heterozygote carrier inheriting TM-QTL from dam; TM^S/TM^D = homozygote carrier

3.3.4 Orthogonal contrasts

Previous work on loin dimensions had shown strong evidence that the mode of inheritance for the TM-QTL deviates from a simple additive model (Macfarlane et al., 2010). Although the results obtained in this study's subset of data did not fully provide the same results, there was certainly an indication for superior loin dimensions in TM-QTL carrier lambs, especially in those with a paternal copy of the TM-QTL. Due to this, sets of orthogonal contrasts were fitted to the genotypes to investigate the situation further. These contrasts allowed testing for any particular patterns in the differences among the TM-QTL genotype (least-squares) means, for loin and spine traits, in order to define if certain modes of gene action may be present.

The first set of orthogonal contrasts was fitted to the genotypes to test for additive, dominance and reciprocal heterozygote models of gene action (Table 3.3; only traits where TM-QTL genotype had a significant effect are shown).

The additive inheritance model was fitted as -1 0 0 1 to the $+^S/+^D$, $TM^S/+^D$, $+^S/TM^D$ and TM^S/TM^D genotypes respectively; testing the difference between the means of the homozygote genotypes. Where the contrast value was positive this showed that TM^S/TM^D had a larger mean than $+^S/+^D$ for that particular trait and vice versa if the contrast value was negative. The difference between $+^S/+^D$ and TM^S/TM^D genotype means was significant for all three loin traits LD_W_LWT, LD_D_LWT and LD_A_LWT, and the spine traits, VN_{THOR}, VN_{LUM} and SPL_{LUM}_LWT. The dominance inheritance model was fitted as -1 1 1 -1 to the $+^S/+^D$, $TM^S/+^D$, $+^S/TM^D$ and TM^S/TM^D genotypes respectively; testing the combined means of the heterozygote genotypes ($TM^S/+^D$, $+^S/TM^D$) with the combined means of the homozygote genotypes ($+^S/+^D$, TM^S/TM^D). However, none of the differences between genotype means were significant, providing no evidence of a dominance effect on any of the traits. The reciprocal heterozygote model of gene action was fitted as 0 1 -1 0 to the $+^S/+^D$, $TM^S/+^D$, $+^S/TM^D$ and TM^S/TM^D genotypes respectively. This contrast tested the difference between the means of the two heterozygote genotypes ($TM^S/+^D$ and $+^S/TM^D$), which were significant for traits LD_A_LWT, VN_{THOR}, VN_{LUM} and SPL_{LUM}_LWT.

Freking et al. (1998a) previously commented that in such a case where the reciprocal heterozygote contrast is shown to be significant, the dominance contrast may be misleading i.e. under and over-estimation of heterozygote genotypes, and further analysis required. Therefore, due to this, and with the previous observation of the TM allele's expression through a non-additive mode of inheritance, a further set of orthogonal contrasts, additive, maternal

dominance and polar overdominance, were fitted to the genotypes as (1 0 0 -1) (-1 0 2 -1) (-1 3 -1 -1), respectively. Results for the additive model have been discussed above, and with no significant results for a maternal dominance effect only the results for the polar overdominance model from this set of contrasts were shown (Table 3.3) and discussed further.

The polar overdominance inheritance model was fitted as -1 3 -1 -1 to the $+^{S}/+^{D}$, $TM^{S}/+^{D}$, $+^{S}/TM^{D}$ and TM^{S}/TM^{D} genotypes respectively and used to test the difference between the mean of the $TM^{S}/+^{D}$ group with each of the means calculated for $+^{S}/+^{D}$, $+^{S}/TM^{D}$ and TM^{S}/TM^{D} genotype groups. Contrast values for the paternal polar overdominance model are the combined differences between genotype means (condition as defined above) and were shown to be significant for all traits tested (Table 3.3); with the exception of VN_{THOR}, $TM^{S}/+^{D}$ genotype lambs had a larger mean compared with each of the other genotype groups.

Table 3.3 Estimates of TM-QTL genotype contrasts (and SE) and significance levels (*P-value*) for additive, dominance, reciprocal heterozygote and paternally derived polar overdominant effects on loin[1] and spine[2] traits.

Trait	Additive			Dominance			Reciprocal heterozygote			Polar overdominance		
	Contrast		*P-value*	Contrast		*P-value*	Contrast		*P-value*	Contrast		*P-value*
LD_W_LWT	1.767	(0.736)	0.017	1.184	(1.151)	0.305	1.368	(0.893)	0.128	3.919	(1.698)	0.023
LD_D_LWT	1.496	(0.697)	0.034	1.142	(1.090)	0.297	1.032	(0.847)	0.225	3.207	(1.609)	0.048
LD_A_LWT	118.9	(46.92)	0.013	15.90	(73.38)	0.829	131.5	(56.98)	0.023	279.0	(108.3)	0.011
SPL_{LUM}_LWT	-5.321	(2.531)	0.037	-1.857	(3.958)	0.640	8.675	(3.073)	0.006	15.49	(5.840)	0.009
VN_{THOR}	0.186	(0.080)	0.022	-0.032	(0.126)	0.800	-0.243	(0.097)	0.014	-0.518	(0.185)	0.006
VN_{LUM}	-0.213	(0.092)	0.022	-0.001	(0.144)	0.994	0.275	(0.112)	0.015	0.550	(0.212)	0.012

First set of orthogonal contrasts was fitted as -1 0 0 1(additive), -1 1 1 -1(dominance) and 0 1 -1 0(reciprocal heterozygote) to the $+^S/+^D$, $TM^S/+^D$, $+^S/TM^D$ and TM^S/TM^D genotypes, respectively. Second set of orthogonal contrasts was fitted as -1 0 0 1(additive), -1 0 2 -1(maternal dominance) and -1 3 -1 -1(polar overdominance) to the $+^S/+^D$, $TM^S/+^D$, $+^S/TM^D$ and TM^S/TM^D genotypes, respectively. From the second set of contrasts, only the results from the polar overdominance test are shown; additive results previously reported with first set of contrasts and the maternal dominance test was not significant for any of the traits.

LWT = live weight (kg) fitted as a covariate in model

[1] LD_W = width of *longissimus thoracis et lumborum* (mm); LD_D = depth of *longissimus thoracis et lumborum* (mm); LD_A = area of *longissimus thoracis et lumborum* (mm^2)

[2] SPL_{THOR} = length of thoracic spine region (mm); SPL_{LUM} = length of lumbar spine region (mm); SPL_{T+L} = length of thoracolumbar spin region (mm); VL_{THOR} = average length of individual thoracic vertebrae (mm); VL_{LUM} = average length of individual lumbar vertebrae (mm); VL_{T+L} = average length of individual thoracolumbar vertebrae (mm); VN_{THOR} = number of thoracic vertebrae; VN_{LUM} = number of lumbar vertebrae; VN_{T+L} = number of thoracolumbar vertebrae

3.4 Discussion

It should be noted that the data set used in the present study was limited in its size, largely due to the restricted availability of sires (identified as TM-QTL carriers) which could be used to produce a study group of lambs. Nonetheless, to date, it is the only available data set which provides detail of the TM-QTL status for a sufficient number of purebred Texel animals, from which, the effects of TM-QTL on carcass, meat quality and production traits could be assessed.

The analysis made use of lamb records, where TM-QTL genotype was unambiguously known, to, (i) determine if similar conclusions for loin dimensions could be formulated using only a subset of data in the analysis, repeating, as close as possible, the model described by Macfarlane et al. (2010), (ii) extend this test to determine if there is an effect of TM-QTL on underlying spine characteristics as the loin muscle is located parallel to spinal vertebrae, and, (iii) fit sets of contrasts to the TM-QTL genotype groups in order to determine the inheritance pattern of the TM-QTL.

It should also be noted that the following discussion will continue to refer only to loin and spine length trait results generated from the model where all lamb records were adjusted for LWT.

3.4.1 TM-QTL and loin traits

Regarding the loin traits, LD_W, LD_D and LD_A, the least-squares means for these traits reported by Macfarlane et al. (2010) are in strong agreement with an overdominance mode of expression of the TM allele; there is evidence of both heterozygote groups lying outside, in this case above, the phenotypic range of the homozygote groups. The results of Macfarlane et al. (2010) even suggested, more specifically, a paternally expressed polar overdominance effect as $TM^{S}/+^{D}$ genotype lambs consistently exceeded $+^{S}/TM^{D}$ genotype lambs in trait means; the difference between heterozygote groups, however, only appeared to be significant for the loin area (LD_A).

From the present Chapter, the polar overdominance test (Table 3.3) did show significance, but the pattern of differences between the $TM^{S}/+^{D}$ genotype least-squares means and the least-squares means for $+^{S}/+^{D}$, $+^{S}/TM^{D}$ and TM^{S}/TM^{D} genotype groups (Table 3.1) conflicts with this outcome and, overall, could not support a polar overdominance mode of TM gene action i.e. trait means for $TM^{S}/+^{D}$ genotype lambs did not appear to significantly ‘out-perform’ over

all (or the majority) of the other genotype groups (Table 3.1). Nonetheless, the pattern of least-squares means did still infer TM expression which could not be explained by simple additive gene action. Though an overdominacne model could not be supported, there was still an indication towards some paternal influence of the TM allele; genotype groups which inherited a copy of the TM allele from the sire ($TM^S/+^D$ and TM^S/TM^D) were observed to have, on average, larger loin width (LD_W_LWT), depth (LD_D_LWT) and area (LD_A_LWT) measures.

3.4.2 TM-QTL and spine traits

Three out of the nine spine traits (VN_{THOR}, VN_{LUM} and SPL_{LUM}) were observed to be significantly different amongst the TM-QTL genotype groups (Table 3.2).

The least-squares means for vertebrae number traits (VN_{THOR}, VN_{LUM}) followed a curious pattern; means for $+^S/+^D$ and $TM^S/+^D$ genotype lambs were similar and significantly different, in most instances, to the means for $+^S/TM^D$ and TM^S/TM^D genotype lambs, the latter of which were also similar to each other in their mean values. The $+^S/+^D$ and $TM^S/+^D$ groups had, on average, fewer thoracic vertebrae (VN_{THOR}) but more lumbar vertebrae (VN_{LUM}), with the situation reversed for the $+^S/TM^D$ and TM^S/TM^D groups. However, the overall number of thoracolumbar vertebrae (VN_{T+L}) across the four genotype groups was not different. It appeared that TM-QTL inheritance patterns had no substantial effect on the total number of thoracolumbar vertebrae but may have some influence on the thoracic-lumbar vertebral arrangement in the spine, but this remains uncertain as the size of difference between genotype groups is small.

Similarly, the total length of the thoracolumbar region (SPL_{T+L}) was not significantly different between the genotype groups. Freking et al. (1998b) observed shorter spinal columns in *CPLG* genotype lambs, and given that TM-QTL falls close to its position on the chromosome, it was an important point to investigate further in connection with TM inheritance. Least-squares means for spine length traits (SPL, VL) from the present chapter, however, do not suggest any such negative effects of TM-QTL on spine length. The $TM^S/+^D$ genotype lambs, which express the muscle hypertrophy phenotype, in fact, were observed to have, on average, longer thoracolumbar vertebrae (VL_{T+L}_LWT), and subsequently longer thoracolumbar spine regions (SPL_{T+L}_LWT), but these trait values (23.22mm; 442.0mm respectively) were not significantly different from the other three groups. It is interesting that in the animals which

were observed to have the largest loin dimension measures ($TM^{S}/+^{D}$), there is not much of a change to the structure on which it lies.

Given that differences in spine traits are largely non-significant between the genotype groups, and that the overall pattern of least-squares means is indistinct, interpreting the models of gene action should be done with reservation. For example, the contrast tests showed significance for the polar overdominant model of TM gene action on VN_{THOR}, VN_{LUM} and SPL_{LUM} spine traits. These results should, again, be carefully considered alongside least-squares means (Table 3.1 & 3.2) as, though slightly larger (for VN_{LUM} and SPL_{LUM}), the means for $TM^{S}/+^{D}$ genotype lambs did not significantly 'out-perform' over all other genotype groups for these spine traits. Hence, there was no strong indication that the observed differences in spine trait phenotypes were associated with increased loin muscling specific to TM gene action.

What is important to take from the present study is that increased loin muscling, particularly associated with $TM^{S}/+^{D}$ genotype lambs, has been shown to have little associated effect on the underlying spine characteristics. Information on spine characteristics, in general, could potentially be used to improve loin production i.e. through increasing the size and/or number of loin chops. Hence, it would be interesting to investigate further the potential size of increase in loin production from those $TM^{S}/+^{D}$ animals which possess a greater number of thoracolumbar vertebrae.

3.5 Conclusion

Given the results from the present study, it was evident that some effect of the TM allele on loin dimension phenotypes was linked to a paternal genetic influence, but, with a weaker data set (67 fewer records) this study could not provide further evidence for a specific polar overdominance inheritance pattern. With regards to spine characteristics, in general terms, the analysis of the subset of data did not reveal any obvious (advantageous or disadvantageous) associations with TM-QTL inheritance. There did not appear to be any effect on spine/vertebrae length and detailing how, or if, the TM allele interacts in the vertebral patterning process (given the thoracic-lumbar vertebral combinations across genotype groups) would require analysing a substantially larger data set than what was available at the time the present study was carried out.

Chapter 4

Estimation of genetic parameters for spine characteristics measured by computed tomography for purebred Texel sheep

4.1 Introduction

The formation, development, and stabilisation of the highly specialised, morphologically and genetically diverse breeds that are recognised amongst the livestock species of today have been due to both natural pressures i.e. changes in the environment, and artificial selection i.e. humans actively selecting the breeding animals on some desirable phenotypic trait(s) (Mignon-Grasteau et al., 2005; Chen et al., 2007; Rubin et al., 2012, Wilkinson et al., 2013). The efficiency of the latter has been greatly improved by collection of performance recordings of traits, and further still, by incorporating quantitative genetics, thus, allowing breeders more accurate predictions of, and greater influence over, the direction and magnitude of single and simultaneous trait modification through selection (Mignon-Grasteau et al., 2005; Chen et al., 2007).

The main carcass/production traits which were of interest throughout this project have been body/carcass size/length, particularly in relation to vertebrae number and length and the overall potential impact from utilising these traits relationship to improve meat yield from sheep. To date, little has been researched concerning the specific area of vertebrae variation to provide a complete picture on this. However, as demonstrated in previous chapters, the increasing use of computed tomography (CT) has moved this forward as it offers the opportunity to measure spine traits *in vivo*, and from doing so has provided evidence of marked intra- and inter-breed vertebral variation (Chapter 2). Still, this has only provided a phenotypic description for spine traits, while estimating their heritability coefficients and exploring genetic relationships with other production traits will allow a more comprehensive understanding to their economic potential to the sheep breeding system.

Studies have indicated body length and vertebrae number in pigs as moderately heritable traits with estimates in the range of 0.50 to 0.54 and 0.60 to 0.62, respectively (Enfield and Whatley, 1961; Fredeen and Newman, 1962b; Duckworth and Holmes, 1968; Borchers et al., 2004). If a similar case exists for sheep, the response to selection and rate of genetic improvement of these (body composition) traits could be rapid through the method of artificial breeding. The aim of the following study is, therefore, to expand on previous results and estimate genetic parameters for spine characteristics, as well as carrying out phenotypic and genetic correlation analysis between spine traits and between spine traits and other CT measured production/carcass traits for purebred Texel sheep, one of the well-established meat breeds and terminal sire lines in the UK sheep industry. This will provide initial insight to the size

and direction of the potential gain to the sheep industry if selective breeding programmes are to include spine traits, and also help determine the level of emphasis to place on such traits for future breed development.

4.2 Materials and Methods

4.2.1 Animal records

The present study used a collection of available purebred Texel lamb records (n = 461). The collection of records included information on trait performance for entire males and females born across the years 2003, 2004 and 2009, from ewes of mixed age. Lambs had been reared as singles, twins or artificially (pet), raised on either a research farm in Scotland (farm 1) or in Wales (farm 2) in the United Kingdom (UK) (Table 4.1); more detailed information on flock management can be found in Lambe et al. (2007), Lambe et al. (2008), Navajas et al. (2008) and Macfarlane et al. (2014).

It should be noted that the Texel population from which the 2009 lamb cohort was produced had been established in a manner so as to investigate the effects of particular muscling genes, specifically, the Texel muscling QTL (TM-QTL), on various production traits relating to the carcass and meat quality. The outcome was lambs born in 2009 were sired by rams that were identified as carriers of at least one copy of TM-QTL; the ewes included carriers and non-carriers of TM-QTL and a number where this was unknown. This allowed classification of a TM-QTL genotype status (homozygote non-carrier, $+^{S}/+^{D}$; heterozygote carrier inheriting TM-QTL from the sire, $TM^{S}/+^{D}$; heterozygote carrier inheriting TM-QTL from dam, $+^{S}/TM^{D}$; homozygote carrier, TM^{S}/TM^{D}) for 142 lambs born in 2009 and could thereby also be included as an effect to be estimated in the present study (details for the genotyping method can be found in Macfarlane et al. 2014). Where any TM-QTL genotype could not be confidently identified for a lamb, they were recorded as unknown for this factor (Table 4.1).

Lambs born in 2003 and 2004 had not been originally genotyped for the TM-QTL, not being part of the selection criteria to produce these lambs, so for the purpose of analysis all lambs born in 2003 and 2004 were treated as missing for the factor TM-QTL genotype (Table 4.1).

Table 4.1 Summary of the distribution of Texel lamb records (n = 461) for fixed factors, arranged by farm.

Farm[1]	Year born			Sex		Rearing rank			TM-QTL genotype[2]					Dam age range (years)
	2003	2004	2009	Male	Female	Single	Twin	Pet	$+^S/+^D$	$TM^S/+^D$	$+^S/TM^D$	TM^S/TM^D	Unknown/Missing	
1	130	124	135	172	217	180	185	24	22	29	14	24	300	2 to 7
2	.	.	72	32	40	61	9	2	17	23	3	10	19	2 to 5
Total	130	124	207	204	257	241	194	26	39	52	17	34	319	

[1] Farm 1 based in Scotland, UK; Farm 2 based in Wales, UK

[2] TM-QTL genotype groups: $+^S/+^D$, homozygote non-carrier; $TM^S/+^D$, heterozygote carrier inheriting TM-QTL from sire; $+^S/TM^D$, heterozygote carrier inheriting TM-QTL from dam; TM^S/TM^D, homozygote carrier

4.2.2 Measurements recorded with the use of computed tomography

The Texel lamb group used in the present study had been previously CT scanned at an average age of 117 days (range 90 – 145 days) and average weight of 34.42 kg (range 16.8 – 48.7 kg); details of the general CT procedure have been previously described in Jones et al. (2002) and Bunger et al. (2011). Importantly, CT has been confirmed as a more advanced, accurate and reliable tool for evaluating an animal's whole body composition *in vivo* (Young et al., 2001). As part of the CT procedure, detailed cross-sectional reference scans (taken through the body at three positions, the ischium bone, the fifth lumbar vertebra and the eighth thoracic vertebra (see Figure 1.8(a))) and topograms (a longitudinal, ventro-dorsal image of the body; (Navajas et al., 2007) (see Figure 1.8(b))) are generated, and from these an extensive range of phenotypic data can be derived. A summary of the CT traits included in the present study, with descriptive statistics, are listed in Table 4.2.

Analysis of CT images were carried out with the use of Sheep Tomogram Analysis Routines software (STAR; version 4.17), developed jointly by Biomathematics and Statistics Scotland (BioSS, Edinburgh, Scotland) and Scotland's Rural College (SRUC, Edinburgh, Scotland). Firstly, pixel analysis of the CT images allows the allocation of each pixel (according to their measured density, in Hounsfield units, and defined density thresholds) to the tissue type, fat, muscle or bone (see section 1.3) (Glasbey and Horgan, 1995; Lambe et al., 2007). This allows area and average densities of these tissue types to be derived for each cross-sectional reference scan and subsequently used to estimate further carcass/body composition traits. Below is a brief summary of what trait information was derived with the use of CT image analysis, more detailed explanations can be found in the references provided.

Measurements recorded for the loin (*longissimus thoracis et lumborum*) included width, depth and area, as analysed from the cross-sectional CT scan taken at the fifth lumbar vertebra (details of general procedures applied to measure loin dimensions can be found in Lambe et al. (2007) and Macfarlane et al. (2014), which closely followed the approach provided by Jones et al. (2002)). Predictions for total weights of carcass fat, bone and muscle were estimated using previously derived breed-specific prediction equations (see Appendix I; the intercepts and coefficients for equations to predict carcass fat, bone and muscle are shown (Macfarlane et al., 2006)). These were developed using measures from three cross-sectional CT reference scans (see section 1.3) and live weight. The prediction equations were calibrated against detailed slaughter and dissection information from a trial Texel sheep population which

included animals of both sexes at a range of ages and live weights to represent the range of animals likely to be CT scanned through selective breeding programmes.

In further note to this, in using these established prediction equations, weights of total carcass fat for 21 lambs were returned as negative values; though not biologically possible it may be reasonable to accept that these lambs were particularly lean, with a carcass fat level which could not be clearly detected in the CT images. As such, these values were kept as negative in the predicted fat data array for the present study; setting to zero i.e. describing the lambs as having no carcass fat is also biologically unlikely and omitting the records for such lean animals was not favoured under the concern that it may unnecessarily truncate the distribution of predicted fat values. The sum of the predicted weights for carcass fat, muscle and bone were also included for each lamb (giving a predicted total tissue, or carcass weight, trait for each lamb). Given all predicted tissue traits and live weight on day of CT scan, tissue proportion traits were also calculated (as described in Table 4.2).

Finally, the topograms of each lamb were analysed in order to measure the length of the thoracic and lumbar spine regions and the number of vertebrae that belonged to each of these spine regions (Chapter 2 describes this protocol in full). Thoracolumbar (thoracic plus lumbar) length and thoracolumbar vertebrae number were calculated as the sum of the thoracic and lumbar lengths and the sum of the thoracic and lumbar vertebrae counts, respectively. These measurements were then used to calculate the average length of individual vertebrae in each spine region i.e. average length of vertebrae in the thoracic spine region was calculated as, thoracic spine length divided by number of thoracic vertebrae, likewise applied to calculate the length of individual vertebrae in the lumbar and thoracolumbar spine regions using the relevant spine length and vertebrae count values.

Table 4.2 Summary of CT[1] derived traits included in study. For each trait, total number of records (N) is provided along with the descriptive statistics, mean, standard deviation (s.d.), minimum (Min.) and maximum (Max.); for vertebrae count traits, median and quartiles 1 and 3 (Q1/Q3) are provided in place of mean and standard deviation.

	Trait[2]	Description	N	Mean	s.d.	Min.	Max.
	CTwt	Live weight on date of CT scanning (kg)	461	32.42	6.037	16.80	48.70
Loin Measurements[3]	LD_W	Width of the *longissimus thoracis et lumborum* (mm)	461	68.83	5.532	50.50	82.00
	LD_D	Depth of the *longissimus thoracis et lumborum* (mm)	461	28.80	4.068	14.00	40.50
	LD_A	Area of the *longissimus thoracis et lumborum* (mm^2)	461	1775	349.9	743.0	2677
Predicted tissue weights	Pfat	Predicted carcass fat weight (kg)	461	1.901	1.308	-0.621	6.861
	Pmusc	Predicted muscle weight (kg)	461	9.792	2.243	3.086	15.91
	Pbone	Predicted bone weight (kg)	461	2.522	0.403	1.277	3.690
	Ptotal	Total predicted tissue weight (kg)	461	14.21	3.704	3.820	24.00
Tissue proportions	Pfat(%)	Predicted carcass fat weight as a percentage of total predicted tissue weight	461	11.85	6.817	-14.21	31.07
	Pmusc(%)	Predicted muscle weight as a percentage of total predicted tissue weight	461	69.64	4.452	55.17	82.28
	Pbone(%)	Predicted bone as a percentage of total predicted tissue weight	461	18.51	3.644	12.37	38.34
	KO(%)	Killing out percentage (total predicted tissue weight as a percentage of CTwt)	461	43.20	4.378	22.74	53.21
	SMY(%)	Saleable meat yield percentage (predicted muscle weight as a percentage of CTwt)	461	30.00	2.636	18.37	35.85
Spine (region) length (SPL)	SPL_{THOR}	Length of $thoracic_{(THOR)}$ spine region (mm)	461	258.3	19.34	198.0	318.0
	SPL_{LUM}	Length of $lumbar_{(LUM)}$ spine region (mm)	461	182.1	13.75	130.0	222.0
	SPL_{T+L}	Length of $thoracolumbar_{(T+L)}$ (thoracic + lumbar) spine region (mm)	461	440.4	25.16	370.0	514.0
Vertebrae length (VL)	VL_{THOR}	Average length of individual $thoracic_{(THOR)}$ vertebrae (mm)	461	20.05	1.400	15.85	24.46
	VL_{LUM}	Average length of individual $lumbar_{(LUM)}$ vertebrae (mm)	461	29.10	1.558	23.86	33.00
	VL_{T+L}	Average length of individual $thoracolumbar_{(T+L)}$ vertebrae (mm)	461	23.00	1.337	19.47	27.05

[1] CT = x-ray computed tomography

[2] Data for all traits was derived from scans produced during CT procedure with the exception of CTwt, which was live weight physically recorded just prior to CT scanning

[3] All measured from the cross-sectional CT scan taken at the fifth lumbar vertebra

Table 4.2 continued. Summary of CT[1] derived traits included in study. For each trait, total number of records (N) is provided along with the descriptive statistics, mean, standard deviation (s.d.), minimum (Min.) and maximum (Max.); for vertebrae count traits, median and quartiles 1 and 3 (Q1/Q3) are provided in place of mean and standard deviation.

	Trait[2]	Description	N	Median	Q1/Q3	Min.	Max.
Vertebrae number (VN)	VN_{THOR}	Number of $thoracic_{(THOR)}$ vertebrae	461	13.00	13/13	12.00	14.00
	VN_{LUM}	Number of $lumbar_{(LUM)}$ vertebrae	461	6.000	6.0/7.0	4.000	7.000
	VN_{T+L}	Number of $thoracolumbar_{(T+L)}$ (thoracic + lumbar) vertebrae	461	19.00	19/19	17.00	21.00

[1] CT = computed tomography

[2] Data for all traits was derived from scans produced during CT procedure with the exception of CTwt, which was live weight physically recorded just prior to CT scanning

4.2.3 Statistical analysis

A mixed-linear sire model with pedigree was fitted to describe live weight (on day of CT scanning) and CT derived traits using restricted maximum likelihood procedures in ASReml (Gilmour et al., 2009). In attempt to fit the most applicable model to the given data, a sire model was used. This was due to inconsistent outcomes generated from analyses with an animal model fitted, with Log-likelihood or parameters unable to converge for a number of runs. Such may have been predominantly due to the structure of the pedigree (see next section).

Preliminary analysis with the sire model was carried out to determine significant influences of fitted fixed effects of farm, with two levels (1, Scotland and 2, Wales), year born, with three levels (2003, 2004 and 2009), sex, with two levels (male and female), rearing rank, with three levels (single, twin and pet), dam age, with six levels (2, 3, 4, 5, 6 and 7 years), and TM-QTL genotype, with four levels ($+^S/+^D$, $TM^S/+^D$, $+^S/TM^D$ and TM^S/TM^D), on live weight and CT derived traits. To note, given the non-normal distribution of the CT derived trait Pfat, analysis was carried out on the square root transformed (as suggested after performing a box-plot test) data array (after a constant of 1 was added to each value).

From the preliminary tests, the fixed effects farm, year born, sex, rearing rank and dam age were shown to be significant over the majority of traits. Consistent with that discussed in Chapter 2, the fixed effect of TM-QTL genotype was found to be significant for the loin traits (LD_W, LD_D, LD_A) but not for any of the spine traits, similarly TM-QTL was not found to be significant for the predicted tissue or tissue proportion traits. The final univariate model for estimating variance components (the contributions of genetic and environmental effects on traits) then included the fixed effects of farm, sex, rearing rank, year born and dam age fitted to all traits, with the addition of the fixed effect TM-QTL genotype fitted to loin traits, in the analyses. Weight of the lamb at time of CT scanning (CTwt) was fitted as a covariate for loin measurement, predicted tissue weight, tissue proportion, spine length and vertebrae length traits (see table 4.2) but was excluded for vertebrae count traits (for reasons as discussed in previous chapters). The genetic effect of sire was fitted as a random factor.

Due to the nature of the data of the vertebrae count traits (VN_{THOR}, VN_{LUM}, VN_{T+L}) it was agreeable to fit them in the model as binary [0, 1] variates i.e. for the vast majority of lambs used in the present study, the vertebrae counts for each spine region would fall into either one of two count categories which were the most common amongst the study group (Figure 4.1).

As there was not a meaningful number of animals which possessed the more "extreme" vertebral counts it was possible to combine categories in order to divide the data into two definite groups, necessary for applying the binomial distribution to the traits. Therefore, for the purpose of analysis, data was divided for each vertebrae count trait as follows:

(1) VN_{THOR}, the two groups comprised of (i) individuals with 12 or less thoracic vertebrae and (ii) individuals with 13 or more thoracic vertebrae
(2) VN_{LUM}, the two groups comprised of (i) individuals with 6 or less lumbar vertebrae and (ii) individuals with 7 or more lumbar vertebrae
(3) VN_{T+L}, the two groups comprised of (i) individuals with 19 or less thoracolumbar vertebrae and (ii) individuals with 20 or more thoracolumbar vertebrae

as shown in Figure 4.1 with the binary groups coded [0, 1]. In the final univariate model, vertebrae count traits were modelled as binomial with the logit link function, for which, the residual variance on the underlying scale is $\frac{\pi^2}{3}$ (~ 3.29) (Gilmour et al., 2009).

Using the variance component estimates provided from the univariate analysis, the measure of heritability (h^2) for traits was calculated as the ratio of the additive genetic variance ($var(A)$: sire variance, $var(S), * 4$) and the total phenotypic variance ($var(P)$: $var(S)$ + residual variance):

$$h^2 = var(A)/var(P)$$

Further to the univariate analysis, bivariate analyses were carried out between all combinations of traits (however, this capability was not available between two binary traits i.e. between the vertebrae count traits). For the bivariate analysis between continuous traits, the model included all fixed effects, covariates, and random effects fitted as they were described for the univariate analysis above. For the bivariate analysis between a continuous and a binary trait, the fixed and random effects were fitted as for the univariate, but no adjustment was made for live weight for either trait.

Phenotypic and genetic correlations between traits were then calculated using the phenotypic and genetic variances and covariances provided by the bivariate analysis. Phenotypic correlations (r_p), between trait 1 (t_1) and trait 2 (t_2), were calculated as the ratio of the total phenotypic covariance ($cov(P)$) between t_1 and t_2 and the square root of the product of the total phenotypic variance of t_1 and the total phenotypic variance of t_2:

$$phenotypic\ correlation = r_p = \frac{cov(P)_{t_1,t_2}}{\sqrt{var(P)_{t_1} * var(P)_{t_2}}}$$

Likewise, genetic correlations (r_g) between t_1 and t_2 were calculated as the ratio of the sire covariance ($cov(S)$) between t_1 and t_2 and the square root of the product of the sire variance of t_1 and the sire variance of t_2:

$$genetic\ correlation = r_g = \frac{cov(S)_{t_1,t_2}}{\sqrt{var(S)_{t_1} * var(S)_{t_2}}}$$

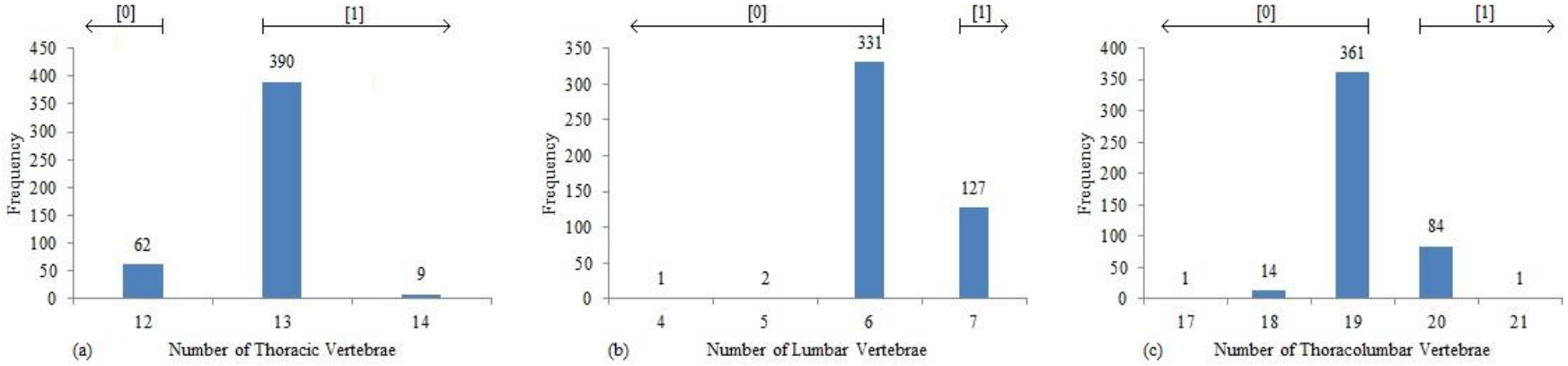

Figure 4.1 Frequency of lambs belonging to each count category for vertebrae number in the thoracic (a), lumbar (b) and thoracolumbar (c) spine region. Count data was divided into two binary groups [0, 1] for analysis.

4.2.4 The pedigree

The Texel sheep pedigree included to complete genetic analysis of traits consisted of a total of 3868 identities over eight generations which consisted of 156 sires and 1239 dams. Of these, the lambs included in the present study were the progeny of 17 sires and 354 dams (Table 4.3). Ten of the sires were used to produce the lambs born in 2003 and 2004 on farm 1; all ten were common over both years, and seven sires were used to produce the lambs born in 2009 on farm 1 and farm 2; three of these sires were common across both farms.

Table 4.3 Number of lambs produced by each sire (by year born and farm) and total number of dams which had been mated (by (lamb) year born and farm).

Sire ID	Year born				Total
	2003 (Farm 1)[1]	2004 (Farm 1)[1]	2009 (Farm 1)[1]	2009 (Farm 2)[2]	
5577	.	.	34	19	53
5623	.	.	.	10	10
6507	.	.	11	.	11
6539	.	.	.	17	17
7765	.	.	22	.	22
805077	.	.	44	12	56
20020040	.	.	24	14	38
20020088	14	9	.	.	23
20020089	20	10	.	.	30
20020100	23	15	.	.	38
20020119	9	14	.	.	23
20020123	15	21	.	.	36
20020157	8	14	.	.	22
20020164	15	12	.	.	27
20020169	7	9	.	.	16
20020455	8	10	.	.	18
20020460	11	10	.	.	21
Total	130	124	135	72	461
Dams mated	89	87	111	67	354

[1] Farm 1 based in Scotland, UK
[2] Farm 2 based in Wales, UK

A particular point to note on this pedigree's structure concerns the high relatedness of the individuals used to produce the 2009 lambs. As mentioned earlier, the design of previous projects meant there was the requirement to increase the frequency of the TM-QTL in the Texel population; hence, both males and females underwent more intensive selection and management in order to increase the frequency of heterozygote TM-QTL carriers and to produce a number of homozygote TM-QTL carriers (Macfarlane et al., 2014).

In light of this, the construction (using Pedigree Viewer (Version 6.5b) (Kinghorn and Kinghorn, 2011)) and visual assessment of pedigree diagrams (e.g. Figure 4.2; not all diagrams shown) revealed that closely related individuals (shared common sires) had been used as parents to produce the 2009 lamb cohort, not only this but the sires common to these particular animals could be traced back to a single sire.

In contrast, it was seen from similar pedigree diagrams produced for the 2003 and 2004 lambs (not included), that these populations of animals had not undergone such strong pre-selection and demonstrated a better example of random mating. Moreover, it was made apparent that due to aims of specific trials, this had resulted in the use of very different parental groups to produce the 2003/04 and 2009 cohorts, thus, effectively splitting the experimental population which resulted in low connectedness between the 2003/4 and 2009 year groups.

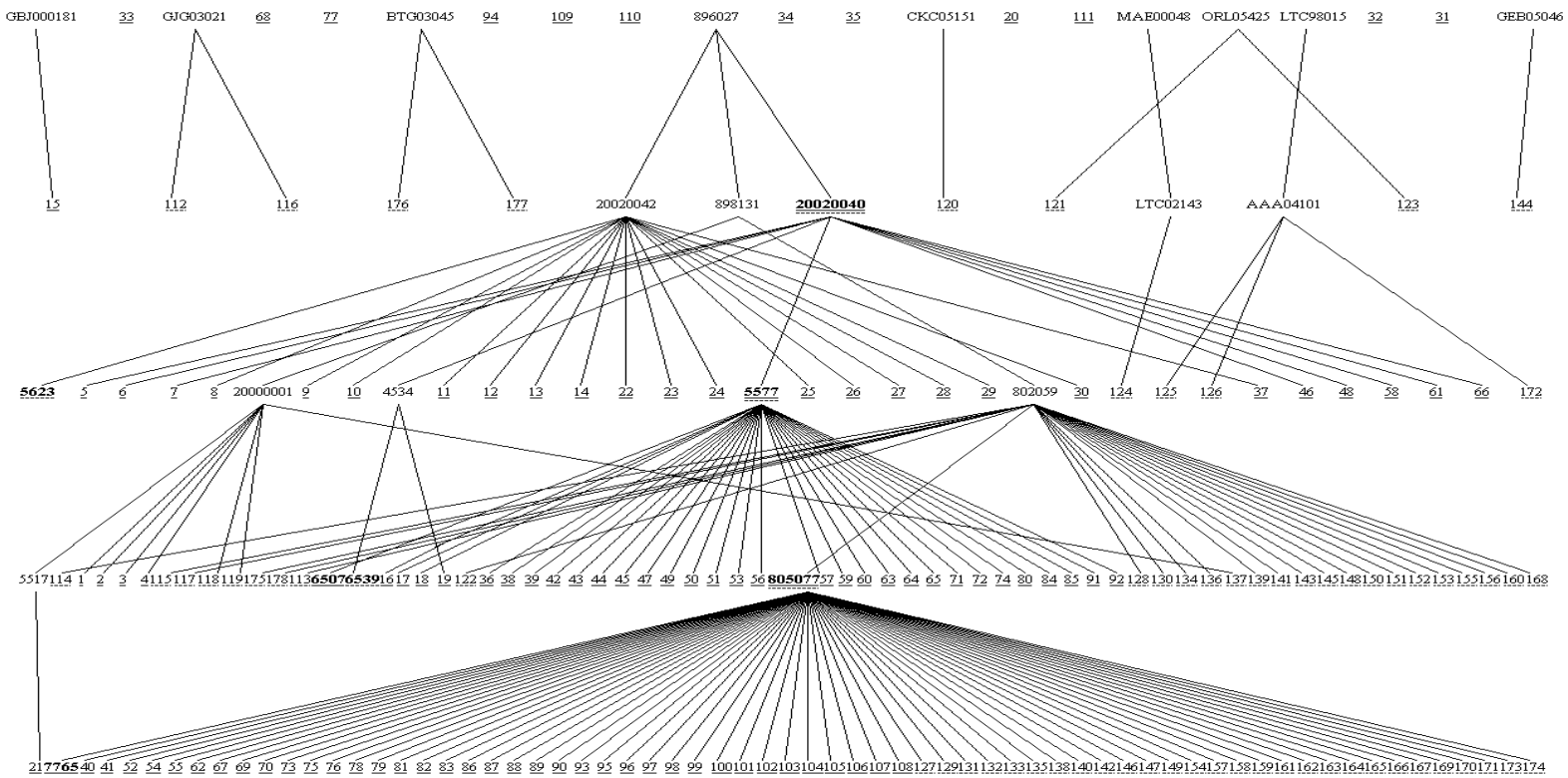

Figure 4.2 A simplified pedigree diagram describing the sire line of sires and dams of the lambs born in the year 2009. Sires of the lambs are in bold and dams of the lambs are numbered consecutively 1 – 178 (for presentation purposes). Sires and dams with a solid underline were used to produce lambs on farm 1 (based in Scotland, UK) and those with a broken underline were used to produce lambs on farm 2 (based in Wales, UK). The dams which appear at the top of the pedigree diagram (with no sire links) are those which had no further sire information available.

4.3 Results

Results contained in Table 4.4 to accompany the following sections (4.3.1 and 4.3.2) have been condensed within this Chapter's text due to the volume of data obtained. The full set of results (with standard errors) can be found in Appendix II.

4.3.1 Univariate analysis: Heritability estimates

Heritability estimates obtained from the univariate analyses for each trait are shown in Table 4.4. Traits ranged widely from low (h^2 = 0.08) to highly (h^2 = 0.99) heritable, but all estimates also had associated high standard errors. This may in part be due to the small data set on which analysis was carried out for the current study. In consideration to this, the results obtained are unreliable in many cases and it was important to compare these estimates with the available literature to highlight where any discrepancies in estimates may have arisen. As far as possible, comparisons were made with studies specifically concerning the Texel breed as well as with previous work from Safari et al. (2005), which involved a review of genetic parameter estimates for a selection of wool, growth, meat and reproduction traits in wool, dual-purpose and meat breeds of sheep.

4.3.1.1 Live weight

Firstly, CTwt (or weaning weight, with lambs at an average age of 16 weeks) was estimated as a highly heritable trait at 0.65. However, this was much higher than previously reported estimates of 0.50 (for body weight), 0.38 (scan live weight) and 0.18 (for weaning weight; 3-5 months of age) reported by Janssens and Vandepitte (2004), Jones et al. (2004) and Safari et al. (2005).

4.3.1.2 Loin measurements

The heritability estimate for LD_W of 0.32 is in good agreement with those reported in literature, 0.39 and 0.30 from Jones et al. (2004) and Janssens and Vandepitte (2004), respectively. Similarly, the heritability estimate for LD_D of 0.30 (Table 4.4) was close to the 0.37 estimate from Jones et al. (2004), but the heritability estimates LD_A were substantially different: 0.69 (Table 4.4) vs. 0.33 (Jones et al., 2004). Safari et al. (2005) had also reported heritability estimates for LD_W, LD_D and LD_A of 0.06, 0.24 and 0.14, respectively, but these were much lower in comparison to the estimates from the current study and other literature; this was particularly evident for the trait LD_W.

4.3.1.3 Predicted tissue weights

Predicted tissue traits Pfat and Pmusc were estimated to have high heritability, 0.42 and 0.50, respectively, and predicted tissue traits Pbone and Ptotal moderate heritability, 0.21 and 0.25, respectively. Again, in comparison with results from Jones et al. (2004), the heritability estimates for the Pfat trait were similar, 0.42 (Table 4.4) vs. 0.40 (Jones et al., 2004), as were the estimates for Pmusc (described as total weight of lean in the carcass in Jones et al. (2004)) with values of 0.50 (Table 4.4) vs. 0.46 (Jones et al., 2004). Ptotal, calculated by summing the predictions of carcass fat, muscle and bone, is effectively an estimate of the animal's carcass weight; with comparisons to such named traits in literature, heritability estimates were observed to be similar 0.25 (Table 4.4) vs. 0.20 (Safari et al., 2005).

4.3.1.4 Tissue proportions

Of the proportion traits analysed, Pfat(%), Pmusc(%) and SMY(%) were estimated to have high heritability, 0.56, 0.47 and 0.34, respectively, and Pbone(%) and KO(%) moderate heritability, 0.26 and 0.18, respectively. Estimation of genetic parameters for proportion traits included in the current study have been less commonly reported on in the literature and, for the majority, comparisons could not be made at this time. With regards to SMY(%), however, it may be acceptable to compare to similar production traits such as lean meat yield (LMY); in this case similar heritability estimates were observed with an estimate of 0.35 reported for LMY (Safari et al., 2005).

4.3.1.5 Spine region length and vertebrae length

Spine (region) length traits were observed to have low to moderate heritability. Estimates for SPL_{THOR}, SPL_{LUM} and SPL_{T+L} were 0.30, 0.08 and 0.14 respectively. A heritability estimate for overall spine length was calculated as 0.46 by Jones et al. (2004), and a similar (body) length trait (as measured from anterior shoulder point to the posterior extremity of the pin bone) was calculated to have a heritability of 0.28 (Janssens and Vandepitte, 2004) which was similar to the heritability estimate (h^2 = 0.27) of body length in Menz sheep (Gizaw et al., 2008). However, all estimates were considerably higher than the 0.14 calculated for SPL_{T+L} in the current study.

As far as it is known, genetic parameters have not before been estimated for the length of the separate thoracic and lumbar spine regions in sheep, nor have they been estimated for the length of individual vertebrae in each spine region. Heritability estimates for the latter traits,

VL_{THOR}, VL_{LUM} and VL_{T+L}, were calculated for the current study and fell within the low and high heritability ranges at 0.55, 0.08 and 0.44, respectively.

4.3.1.6 Vertebrae number

Heritability estimates for VN_{THOR}, VN_{LUM} and VN_{T+L} were 0.99, 0.08, and 0.44, respectively. Again, in sheep, genetic parameters have not before been estimated for vertebrae number traits.

4.3.2 Bivariate analysis: Phenotypic and genetic correlations

Firstly, phenotypic and genetic correlations between the spine traits will be briefly reviewed. Following will be the findings considering the associations between the spine traits and production traits. Given the aims of the present study, correlations between production traits are not considered at this time. It should be noted that, similar to the outcome of the heritability estimates, calculated standard errors are large relative to the correlation estimates obtained, particularly for the genetic correlations, so for many cases the results are not reliable. Their accuracy could be improved by including additional data when available.

4.3.2.1 Correlations between spine traits

Highly positive correlations between SPL_{THOR} and VL_{THOR} (r_p = 0.81; r_g= 0.72) and between SPL_{T+L} and VL_{T+L} (r_p = 0.73; r_g= 0.76) suggest that there was an associative increase in the length of these spine regions with an increase in the length of the vertebrae in the respective spine regions, rather than by number of vertebrae; phenotypic and genetic correlations between SPL_{THOR} and VN_{THOR} and between SPL_{T+L} and VN_{T+L} were r_p = 0.13; r_g= -0.23 and r_p = 0.05; r_g= -0.43, respectively. In addition, correlations between VL_{THOR} and VN_{THOR} (r_p = -0.13; r_g= -0.63) and between VL_{T+L} and VN_{T+L} (r_p = -0.17; r_g= -0.77) were all negative, therefore, an increase in the number of vertebrae in the thoracic or across the thoracolumbar spine region appeared to be associated with a shorter length of these vertebrae, particularly suggestive of the moderate to high estimates for the genetic correlations.

In contrast, low estimates for correlation coefficients between SPL_{LUM} and VL_{LUM} (r_p = -0.01; r_g= 0.29) but higher estimates for correlation coefficients between SPL_{LUM} and VN_{LUM} (r_p = 0.39; r_g= 0.83) suggest that there was an associative increase in the length of the lumbar spine region with an increase in the number of vertebrae. In addition, correlations between VL_{LUM} and VN_{LUM} (r_p = -0.15; r_g= 0.09) indicated low, if any, association between the traits, therefore

indicating that length of the individual lumbar vertebrae were little affected by how many vertebrae comprised the lumbar spine region.

4.3.2.2 Correlations between spine traits and production traits

To note, analysis between some combinations of traits failed due to parameters unable to converge, therefore, no correlation coefficients could be produced. In general terms, positive and negative phenotypic and genetic correlations were observed across all spine and production trait combinations analysed; the strength of the phenotypic correlations were either very small or none was apparent (r_p = 0.00 to 0.20), on the other hand, the strength of the genetic correlations observed across the same trait combinations ranged more widely, from where no correlation was evident to very highly correlated (r_g = 0.00 to 0.99). Below, the genetic associations between traits were explored further.

Firstly, in the assessment of genetic correlations between spine and loin traits, spine traits of the lumbar spine region (SPL/VL/VN_{LUM}) appeared to have consistently strong associations (r_g = 0.46 to 0.99) with LD_W, LD_D and LD_A; these correlations were negative between loin measurements and spine traits SPL_{LUM} and VN_{LUM} and positive between loin measurements and VL_{LUM}. Positive correlations to a similar degree (r_g = 0.28 to 0.75) were observed between loin measurements and spine traits SPL_{THOR} and SPL_{T+L}, however the strength of the correlations between spine traits VL_{THOR}, VL_{T+L}, VN_{THOR} and VN_{T+L} and loin measurements fell considerably (r_g = 0.05 to 0.37).

In the assessment of genetic correlations between spine traits and predicted tissue weight traits, positive correlations were observed between SPL_{THOR} and tissue weight traits Pmusc, Pbone and Ptotal (r_g = 0.76, 0.40 and 0.42, respectively), while a negative correlation was observed between SPL_{THOR} and Pfat (r_g = -0.55). The opposite was observed between SPL_{LUM} and the tissue weight traits; correlations between SPL_{LUM} and Pmusc, Pbone and Ptotal were negative (r_g = -0.76, -0.24 and -0.37, respectively) and the correlation between SPL_{LUM} and Pfat was positive (r_g = 0.94). However, when considering SPL_{T+L} and tissue weight traits, correlations, if any, were positive but low between SPL_{T+L} and Pfat, Pmusc and Ptotal (r_g = 0.06, 0.36, and 0.27, respectively) and moderately negative between SPL_{T+L} and Pbone (r_g = -0.61). Overall, the degree of correlation, again if any, was low (r_g = 0.00 to 0.49) between vertebrae length traits (VL_{THOR}, VL_{LUM}, VL_{T+L}) and tissue weight traits and, generally, negatively associated. Those correlations which could be estimated between predicted tissue weight traits and

vertebrae number traits (VN_{THOR}, VN_{LUM}, VN_{T+L}) were also observed to be low in degree (r_g = 0.12 to 0.50) and all negatively associated.

Finally, the assessment of genetic correlations between spine traits and tissue proportion traits revealed no particular pattern to the size and/or direction of their associations. Moderate to high positive correlations were observed between SPL_{THOR} and traits Pmusc(%), KO(%) and SMY(%) (r_g = 0.65, 0.93, 0.98, respectively), while low to moderate negative correlations were observed between SPL_{THOR} and Pfat(%) (r_g = -0.31), and between SPL_{THOR} and Pbone(%) (r_g = -0.57). All tissue proportion traits other than Pfat(%) were observed to have a negative association with SPL_{LUM} with correlations ranging from a very low to high degree (r_g = 0.23 to 0.80); Pfat(%) was highly positively correlated with SPL_{LUM} (r_g = 0.89). Whereas, all tissue proportion traits other than Pbone(%) were observed to have a positive association with SPL_{T+L} with correlations ranging from a very low to very high degree (r_g = 0.22 to 0.96); Pbone(%) was moderately negatively correlated with SPL_{T+L} (r_g = -0.63). Little association was observed between the majority of tissue proportion traits and vertebrae length traits (VL_{THOR}, VL_{LUM}, VL_{T+L}), with the degree of correlations, where any evident, ranging positively from very low to low (r_g = 0.12 to 0.48). This did not apply to Pbone(%), which was negatively correlated with VL_{THOR} (r_g = -0.68) and VL_{T+L} (r_g = -0.87). The majority of correlations between the vertebrae number traits (VN_{THOR}, VN_{LUM}, VN_{T+L}) and the tissue proportion traits were low or of a level that was too low to suggest any associations.

Table 4.4 Estimates of phenotypic (above diagonal) and genetic (*below diagonal*) correlation coefficients between CT-derived traits and heritability estimates[1] (**on diagonal**). Values are presented in an abbreviated format to allow readability in the available space; see Appendix II for full data.

Trait	CT wt	LD_W	LD_D	LD_A	Pfat	Pmusc	Pbone	Ptotal	Pfat(%)	Pmusc(%)	Pbone(%)	KO(%)	SMY(%)	SPL_{THOR}	SPL_{LUM}	SPL_{T+L}	VL_{THOR}	VL_{LUM}	VL_{T+L}	VN_{THOR}	VN_{LUM}	VN_{T+L}
CTwt	**.65**	.79	.73	.81	.93	.95	.92	.98	.87	-.75	-.81	.82	.45	.73	.48	.82	.75	.81	.83	-.04	-.04	-.06
LD_W	*.85*	**.32**	.23	.60	-.15	.47	.27	.30	-.08	.27	-.26	.42	.50	.12	.10	.19	.04	.08	.07	.09	-.03	.03
LD_D	*.83*	*.84*	**.30**	.81	.03	.53	.06	.49	.05	.21	-.40	.54	.55	.02	-.16	-.12	-.02	-.08	-.08	.02	-.08	-.05
LD_A	*.74*	*.89*	*.98*	**.69**	.00	.63	.13	.56	.01	.26	-.40	.60	.63	.08	-.11	-.02	-.04	-.00	-.07	.08	-.08	-.01
Pfat	*.97*	*-.25*	*.19*	*.13*	**.42**	-.29	-.34	.40	.93	-.84	-.38	.34	-.22	-.09	-.02	-.10	-.11	-.06	-.12	NE^2	NE^2	NE^2
Pmusc	*.96*	*.75*	*.38*	*.52*	*-.74*	**.50**	.22	.70	-.28	.63	-.44	.73	.95	.10	-.15	-.04	.03	-.06	-.03	-.01	-.07	-.05
Pbone	*.97*	*.41*	*.50*	*.29*	*-.70*	*.60*	**.21**	.13	-.27	.08	.35	.21	.24	.20	-.00	.15	.21	.12	.20	NE^2	NE^2	NE^2
Ptotal	*.99*	*.76*	*.65*	*.77*	*-.27*	*.82*	*.38*	**.25**	.27	-.02	-.45	.87	.67	.02	-.17	-.14	-.05	-.14	-.12	-.02	-.05	-.05
Pfat(%)	*.93*	*-.19*	*.16*	*.07*	*.95*	*-.71*	*-.52*	*-.36*	**.56**	-.82	-.53	.40	-.13	-.03	.00	-.02	-.04	.02	-.03	-.03	-.01	-.06
Pmusc(%)	*-.88*	*.41*	*-.14*	*.06*	*-.93*	*.89*	*.48*	*.51*	*-.93*	**.47**	-.05	.01	.57	.07	-.04	.03	.05	-.00	.03	.04	-.01	.04
Pbone(%)	*-.93*	*-.28*	*-.06*	*-.26*	*-.54*	*.04*	*.34*	*-.08*	*-.67*	*.35*	**.26**	-.70	-.61	-.05	.05	.02	-.01	NE^2	.00	.01	.04	.07
KO(%)	*.94*	*.94*	*.70*	*.86*	*-.04*	*.62*	*.38*	*.73*	*.10*	*.19*	*-.58*	**.18**	.82	.12	-.14	-.03	.04	NE^2	-.01	.03	-.07	-.05
SMY(%)	*.44*	*.86*	*.36*	*.56*	*-.61*	*.96*	*.57*	*.79*	*-.51*	*.75*	*-.20*	*.78*	**.34**	.13	-.13	.00	.07	.00	.02	.07	-.06	-.03
SPL_{THOR}	*.85*	*.75*	*.56*	*.53*	*-.55*	*.76*	*.40*	*.42*	*-.31*	*.65*	*-.57*	*.93*	*.98*	**.30**	-.37	.60	.81	.39	.69	.13	-.23	-.08
SPL_{LUM}	*.81*	*-.51*	*-.60*	*-.46*	*.94*	*-.76*	*-.24*	*-.37*	*.89*	*-.80*	*-.68*	*-.23*	*-.68*	*-.65*	**.08**	.52	-.13	-.01	.11	-.19	.39	.22
SPL_{T+L}	*.95*	*.59*	*.28*	*.42*	*.06*	*.36*	*-.61*	*.27*	*.25*	*.22*	*-.63*	*.96*	*.73*	*.85*	*-.16*	**.14**	.64	.34	.73	-.01	.05	.05
VL_{THOR}	*.84*	*-.20*	*.19*	*-.05*	*-.24*	*.28*	*-.00*	*-.05*	*.01*	*.29*	*-.68*	*.43*	*.48*	*.72*	*-.42*	*.71*	**.55**	.39	.94	-.13	-.13	-.21
VL_{LUM}	*-.11*	*.99*	*.78*	*.87*	*-.02*	*.49*	*-.44*	*.42*	*.15*	*.31*	NE^2	NE^2	*.85*	*.89*	*.29*	*.62*	*.61*	**.08**	.61	.01	-.15	-.13
VL_{T+L}	*.90*	*-.20*	*.17*	*-.06*	*-.12*	*.21*	*-.23*	*-.08*	*.12*	*.22*	*-.87*	*.48*	*.44*	*.68*	*-.25*	*.76*	*.99*	*.67*	**.44**	-.13	-.08	-.17
VN_{THOR}	*-.42*	*.27*	*-.10*	*.12*	NE^2	*-.28*	NE^2	*-.35*	*-.40*	*.38*	*.39*	*-.15*	*.24*	*-.23*	*-.42*	*-.34*	*-.63*	*-.38*	*-.61*	**.99**	NA	NA
VN_{LUM}	*-.04*	*-.81*	*-.63*	*-.65*	NE^2	*-.51*	NE^2	*-.12*	*.45*	*-.57*	*-.13*	*-.34*	*-.26*	*-.68*	*.83*	*-.19*	*-.33*	*.09*	*-.13*	*NA*	**.08**	NA
VN_{T+L}	*-.33*	*.13*	*-.37*	*-.08*	NE^2	*-.31*	NE^2	*-.28*	*-.27*	*.15*	*.43*	*-.22*	*-.15*	*-.35*	*-.28*	*-.43*	*-.86*	*-.20*	*-.77*	*NA*	*NA*	**.44**

[1] Heritability estimates taken from the univariate analysis

[2] Model could not converge, parameters not estimable (NE)

4.4 Discussion

The genetic improvement of livestock can and has resulted in large economic returns. However, information from the genetic evaluation of traits has been more widely incorporated in some species (e.g. pig) breeding systems over others (e.g. sheep). For instance, the performance of commercial pig breeds (e.g. Landrace, Large White, Duroc) for meat production and quality has been improved by the simultaneous (genetic) manipulation of a wealth of economically important meat and carcass traits such as growth rate, carcass composition, body size, lean meat content and muscularity (Rubin et al., 2012; Wilkinson et al., 2013).

Of particular interest to the current study was the long-term intensive selection on body size, or more specifically body/carcass length, in pigs which has resulted in the pig body becoming progressively longer (Fan et al., 2013; Wilkinson et al., 2013). As mentioned in earlier chapters, body length is associated with vertebrae number; it is in fact almost completely determined by the number of thoracolumbar vertebrae and their individual lengths (King and Roberts, 1960; Berge, 1984; Mikawa et al., 2011). So with the elongation of the pig body, there has also been a parallel increase in the number of vertebrae over time (Rubin et al., 2012; Fan et al., 2013).

Moreover, pig populations in which body length/vertebrae number has increased have proved to be economically superior with larger carcasses and increased meat production (Shaw, 1930; Mikawa et al., 2011; Ren et al., 2012). Evidence has now also been presented which suggests some positive phenotypic associations between spine traits and other important production traits in sheep breeds/crosses (Chapter 2). With the above, the further genetic evaluation of spine traits in sheep was encouraged. The current study approached the genetic analysis of spine traits in the Texel breed of sheep by estimating their genetic parameters (variance components, heritability) and their (phenotypic and genetic) relationship with other important traits.

4.4.1 Heritability estimates

Heritability estimates for CTwt, loin traits, predicted tissue weight traits and tissue proportion traits fell in the moderate to highly heritable range (h^2 = 0.18 to 0.69). The heritability estimates calculated in the present study that could be compared with those presented in literature were in close agreement. The few discrepancies between heritability estimates from

the present study and literature were, however, fairly substantial i.e. CTwt and loin traits. Safari et al. (2005) reported much lower heritabilities for LD_W, LD_D and LD_A but a possible explanation for this may be the different methods used for measuring these traits; ultrasound (Safari et al., 2005) vs. CT scanning (Jones et al., 2004; current study).

As far as it was known, no studies concerning sheep have estimated genetic parameters for a number of the spine traits of interest included in the present study. Length of the thoracolumbar spine region (SPL_{T+L}) was the only spine/vertebrae length trait comparable to the literature (regarding sheep). The heritability calculated for SPL_{T+L} in the present study (h^2 = 0.14) was observed to be much lower than other previous estimates for spine/body length in sheep (e.g. h^2 = 0.46; Jones et al., 2004). In comparison to other livestock species, the heritability estimate of 0.14 remained comparably low; the same trait in pigs was calculated to have a heritability of ~ 0.50 (Enfield and Whatley, 1961).

In consideration of all spine/vertebrae length traits, it was interesting to observe that the degree of heritability for these traits was noticeably higher for the thoracic region over the thoracolumbar region, and more so over the lumbar region i.e. the heritability estimate for SPL_{THOR} (h^2 = 0.30) was higher than the heritability estimate for SPL_{T+L} and SPL_{LUM} (h^2 = 0.14 and 0.08, respectively), similarly, the heritability estimate for VL_{THOR} (h^2 = 0.55) was higher than heritability estimate for VL_{T+L} and VL_{LUM} (h^2 = 0.44 and 0.08, respectively). Such highlights that, if there was selection specifically on the thoracic spine region, genetic progress would be much faster for this trait than if there was specific selection for a longer lumbar spine region. It may be that this situation would be a useful option to breeders/producers in some instances, but given the intermediate heritability estimate for SPL/VL_{T+L}, which are also still of a moderate (0.14) to high degree (0.44), it may be, for a more general approach, the optimum to select on these thoracolumbar spine measurements to improve performance in spine/carcass length.

A similar situation was observed for the heritability estimates for vertebrae number traits, whereby the heritability estimate for VN_{THOR} (h^2 = 0.99) was much higher than the heritability estimate for VN_{T+L} and VN_{LUM} (h^2 = 0.44 and 0.08, respectively). Such an extremely high heritability estimate for VN_{THOR} was unexpected and it was considered that obtaining such a high estimate may have been influenced by the distribution of thoracic-lumbar vertebral formula in progeny across sires (presented in Table 4.5) i.e. was there evidence to suggest that the variation in thoracic-lumbar vertebral formula of progeny between sires was high relative

the variation within sire? This may have inflated the variance estimate for the genetic (sire) variance component. However this could not be confirmed (Table 4.5). Overall there was little variation for thoracic vertebrae number and this was apparent for both within and between sires. It would have been interesting to also have note of the thoracic-lumbar vertebral formula of the sires (and dams) to further extrapolate how theirs contributes to the progeny's (thoracic-lumbar) vertebral formula (e.g. such is illustrated by Fredeen and Newman (1962b)) to give such frequency distributions (Table 4.5).

No heritability estimates have been previously reported for vertebrae number in sheep, but these traits have been investigated in pigs (e.g. Fredeen and Newman, 1962b; Borchers et al., 2004). Heritability estimates reported for the trait VN_{THOR} in these studies (h^2 = 0.73 (Fredeen and Newman, 1962b) and 0.51 (Borchers et al., 2004)) are of a much lower degree compared to that obtained in the present study. It may be fair to assume that the heritability of vertebrae number traits would be similar in sheep. Therefore, the unexpected estimates obtained (e.g. for VN_{LUM} (h^2 = 0.08) as well as VN_{THOR} ()) in the present study are most likely associated with the small number of animals in the data set and the structure of the pedigree (see Section 4.2.4). Combined, this was not an ideal design to provide grounds for robust genetic analysis, particularly due to the reduced variance in the base population. In fact, this is true for the majority of genetic parameter estimates (heritability, above, and genetic correlations, discussed below) obtained from the current study due to the high standard errors, relative to the estimates, calculated. The reliability of the results is therefore low and their accuracy would be improved by including additional data where available.

Table 4.5 Distribution of lambs by their thoracic-lumbar vertebral formula, within sire.

	Thoracolumbar vertebrae number (17) – (21) and Thoracic(T)-Lumbar(L) vertebral formula								
	(17)	(18)		(19)		(20)		(21)	Total
Sire	13T 4L	12T 6L	13T 5L	12T 7L	13T 6L	13T 7L	14T 6L	14T 7L	
5577		1		6	42	4			53
5623					9	1			10
6507					10	1			11
6539		1		2	11	3			17
7765		1			20	1			22
805077		1		5	47	3			56
20050040		3	2	9	23	1			38
20020088				1	17	2	3		23
20020089		1		2	18	7	1	1	30
20020100		1		10	23	4			38
20020119	1	1			16	5			23
20020123				1	23	12			36
20020157		2		9	11				22
20020164				2	12	12	1		27
20020169					9	5	2		16
20020455				1	10	6	1		18
20020460				2	10	9			21
Total	1	12	2	50	311	76	8	1	461

4.4.2 Phenotypic and genetic correlations

4.4.2.1 Correlations between spine traits

To summarise, the majority of the correlations between spine region and vertebrae length traits ranged from low to very high in strength and were generally positive. Correlations between the length traits and vertebrae number traits were all low in strength and generally negative. The genetic correlations between all combinations of spine traits remained in the same direction as their phenotypic associations but were generally higher and with much higher standard errors. From the results obtained in the present study, similar conclusions could be drawn as those reported in Chapter 2. These included (i) increased length of the thoracic spine region seemed to be predominantly achieved through longer individual vertebrae (rather than additional vertebrae), and (ii) increased length of the lumbar spine region seemed to be predominantly achieved through additional vertebrae (more so than through the individual vertebrae being longer). This information is valuable in order to tailor selection decisions if there is the aim to improve the length of a specific spine region. However, it should be noted that correlations between the thoracic and lumbar length traits imply that the general positive effect on one spine region would be at the 'expense' of the other i.e. SPL_{THOR} and SPL_{LUM} were observed to be negatively correlated (r_p = -0.37; r_g= -0.65), thus providing a net effect of zero, effectively.

4.4.2.2 Correlations between spine traits and production traits

Phenotypic correlations between spine traits and production traits were of such a small degree that they were negligible. The genetic correlations for the same spine and production trait combinations were higher but again with high standard errors. From previous analyses there has been some suggestion of positive phenotypic associations between spine traits and some production traits (Chapter 2). However, from the results of the present study, it is difficult to distinguish any specific pattern of directional associations between these groups of traits to support this further.

Overall, it appeared that any change in vertebrae length traits or vertebrae number traits were not strongly associated with any change in production traits. With regards to the correlations between spine length traits and production traits, some crude conclusions can be made. For example, loin traits were negatively associated with SPL_{LUM} and VN_{LUM} but positively associated with VL_{LUM}. In keeping with the suggested relationships between spine traits, as described above ((i) a longer lumbar spine region is associated with an increase in the number of lumbar vertebrae, (ii) a shorter lumbar spine region is associated with longer individual

lumbar vertebrae, and (iii) an increase in the number of lumbar vertebrae is associated with a reduction in length of the individual lumbar vertebrae), these correlations then indicated that an increased length in the lumbar spine region was associated with a decreased loin width, depth and area. Such was a running observation with the other production traits whereby economically favourable traits e.g. Pmusc, KO%, SMY%, were observed to have a negative association with an increased length in the lumbar spine region.

These same production traits were observed to be positively associated with the length of the thoracic spine region. However, when considering the correlations between spine traits of the thoracolumbar region and production traits, for example, predicted tissue weights, the correlations were much lower in strength. This suggests that any improvement made in one spine region, for either its own characteristics (e.g. length) or in relation to an improvement in associated production traits (e.g. increased muscle), is removed when considering the combined effects in each of the spine regions.

4.5 Conclusion

Heritability estimates obtained from the present study were extremely variable in degree across spine traits, ranging from low (h^2 = 0.08; VL_{LUM} and VN_{LUM}) to very high (h2 = 0.99; VN_{THOR}). Variation was observed in vertebrae number so there is the potential for selection, but from this study the proportion contributed to by genetics could not be confidently determined. Optimally, a much larger number of observations are required to obtain heritability estimates with acceptably small standard errors (i.e. acceptable accuracy). Moreover, the pedigree used in the present study was also small and limited further due to missing information on relatives and few sire groups. Thus, leading to less accurate estimates for heritability of traits and genetic correlation coefficients, and in turn, also reducing the confidence in these estimates. Nonetheless, the study has widened the knowledge and information available on spine traits. This can hopefully help in developing future conclusions on the use of these traits in selective breeding for genetic improvement and breed development in sheep.

Chapter 5

General discussion

Through the process of selective breeding in livestock species, numerous economically important traits are commonly manipulated to achieve a specific breeding goal or set of breeding objectives. The principle of this process is to select a proportion of animals (stronger selection pressure on sires) to become parents that will improve the genetic level for these economically important traits in the next generation the most (Strandberg and Malmfors, 2006a, 2006b). The basis for identification and selection of high genetic merit animals firstly includes taking a measure of the animal's individual performance and that of their relatives to obtain a heritability (h^2) estimate (proportion of the total phenotypic variation attributable to additive genetic variation) for each trait of interest, within breed. It is then possible to estimate the breeding value (EBV) for each potential parent using the heritability estimate and the phenotypic value (as a deviation from the genetic base to which the animal is compared) of each individual. This provides a prediction to what proportion of superiority of the parents for each trait, on average, will be passed to the offspring and the average increase in performance of the next generation for that trait (Falconer and Mackay, 1996).

The estimated breeding values can be further used in selection index methods. A selection index allows the simultaneous selection of important traits which the breeder wishes to genetically improve for a specifically chosen breeding goal. However, their use also means that other important traits, which may not be directly associated to any one particular breeding goal, are not excluded. For example, current market demand places pressure on producing lean meat, and selection indices allow breeders and farmers to select sires with high genetic merit for these traits but also allow assessment of, or simultaneous selection for, other traits so that the next generation does not under perform for these traits. This is an important feature particularly for traits such as prolificacy.

Despite the use of selection indices by some sheep breeds and systems, on the whole, the sheep industry is not in such a strong position as the cattle and pig industries. There is still a high incidence of the practice of using simple visual assessment, with little widespread introduction and/or uptake of technological methods, and there is still inconsistent levels of performance recording within and across contemporary groups. The key issue is that when selecting livestock for breeding, it is important to (continue to) use visual assessment but in combination with EBVs and a selection index that reflects the breeding goal. It is therefore important that the sheep industry improve on this current situation through improved production efficiency which translates to better financial returns, particularly as farmers move away from sheep breeding to pursue other areas with the potential for higher profit. A small recovery has been

made over the past few years. In order to continue in this direction and remain as one of the top producers of sheep meat, broader breeding goals which include a wider array of traits may be in accordance to increasing profitability for the sector as well as encouraging product demand.

The research presented in this thesis was carried out with the aim to investigate the potential of exploiting new traits in the selective breeding of sheep in response to an enquiry from terminal sire sheep breeders. In these sheep breeds one of the main points for selection to date has been visually assessed gigot size. However, this selection has meant that in some animals there is a perceived tendency for the length of the high valued loin to be lower than would be commercially desirable. Therefore, the traits of interest here were concerned with characteristics (spine region length, vertebrae length, vertebrae number) of the thoracic, lumbar and thoracolumbar (thoracic plus lumbar) regions of the spine, and their potential contribution to the improvement of overall performance for body/carcass/loin length in sheep stock, under the notion that meat yield/quantity of meat cuts (e.g. loin chops) may be increased.

The present study has been conducted using data from Texel animals as they are the most numerous and widely used terminal sire breed in the UK (and as a result also have the most CT data available). However, the data presented would also be likely to be relevant to other terminal sire breeds (especially the Beltex, which is derived from Texel), although further specific information will be required in the future for breeders of terminal sire and other breeds of sheep to capitalise on the findings presented in this study. Since the study shows that a selection opportunity exists in regards to spine characteristics, within Texel and the other sheep breed/crosses included in the study, this would be in line with increasing the efficiency of production, presenting the prospect of an added cut-out value for each slaughter lamb. In turn, this may allow the cost of their produce to be more competitively priced (Simm, 1998) and increase desirability to consumers i.e. value for money.

The evaluation of spine traits firstly included assessing the extent of their variation and confirming that the measurement method which was used to do so was sound. For the four sheep genotypes (Texel, Scottish Blackface, Texel cross Mule and Poll Dorset cross Mule) in which this was investigated, significant variation was observed within and between genotypes for each of the spine traits. However, the Texel breed was the main focus throughout this project (and was selected for further evaluation of spine traits) so discussion on these results will centre on this breed.

Texel is one of the well-established meat breeds in the UK and the rams are commonly used as terminal sires to produce slaughter lambs for market. Moreover, it is in these 'elite' terminal sire breeds where the most significant proportion of genetic improvement is targeted, therefore it is most suitable to determine the nature of new traits in these breeds as selection intensity would be much higher, if economically valuable. Interestingly, the Texel was found to have the largest within-breed range for thoracolumbar vertebrae number (17 – 21; the majority possessing 19), but the spine length of these animals was, on average, significantly shorter than the other groups.

The method used in order to carry out this assessment was X-ray computed tomography (CT). This procedure produces a number of scans of the animal, the topogram being the primary scan used to measure spine traits (Figure 5.1). With high reproducibility of spine measurements between operators, in this respect, CT was confidently accepted as a reliable method to quantify spine characteristics. However, in later review of this method, there was some question as to whether taking spine measures from this scan alone is under or over estimating the lengths of spine regions i.e. by ignoring the curvature of the spine and any effect which muscling may have on the positioning of the animal for CT scanning (more in relation to terminal sire breeds). Such effects would be missed by assessing only the topogram scan displaying the coronal plane of the animal (Figure 5.1).

As part of a study to investigate this, Bunger et al. (2014) carried out correlation (and regression) analysis between (thoracic, lumbar and thoracolumbar) spine region length measurements taken from the coronal (division of animal in dorsal-ventral halves) and sagittal (division of animal in left-right sides) scans (Figure 5.1) for three major terminal sire breeds (Charollais, Suffolk, and Texel). Hence, correlation was estimated between the length of the lumbar spine region measured from the coronal scan and the length of the lumbar region measured from the sagittal scan, and so forth for each spine region; this was analysed within breed, results shown in Table 5.1 (adapted from Bunger et al. (2014)).

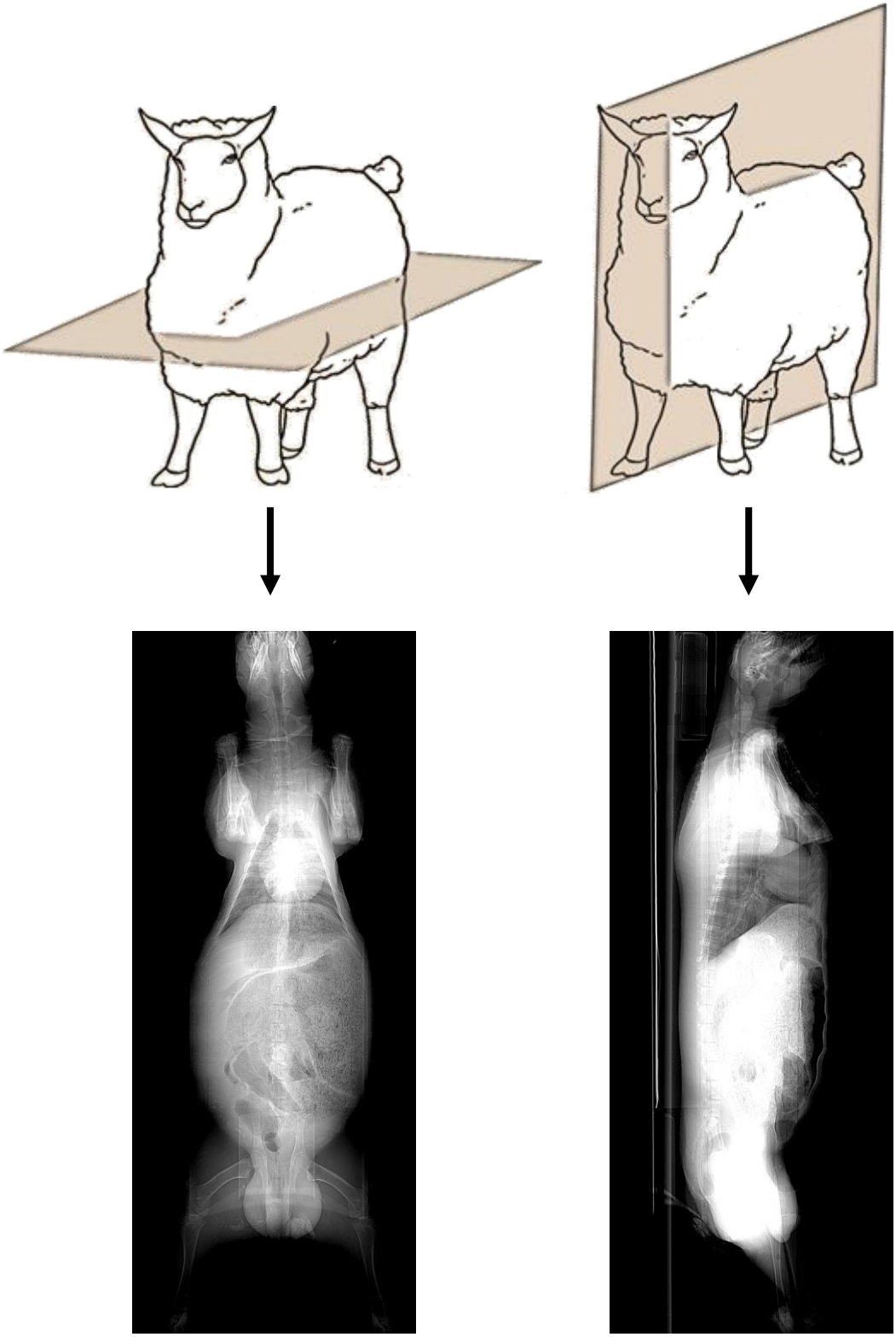

Figure 5.1 Togograms displaying the coronal (left) and sagittal (right) plane of the animal. The coronal plane divides the animal into dorsal and ventral halves and the sagittal plane divides the animal into left and right sides.

Correlations between topogram type (coronal and sagittal) for the spine region length measures were lowest for Charollais, highest in Suffolk and intermediate for Texel. The degree of some of the correlation estimates provides evidence that there can be substantial discrepancies between the coronal and sagittal measurements for spine region length. However, this appears to be a more significant issue for the Charollais breed than the Suffolk or Texel breed. Nonetheless, this has left the question as to whether both types of scan should be used in the future to refine the measurements for the spine region length phenotype, assuming that both can be made available. However, there can be difficulty in defining the boundaries between spine regions on the sagittal scans, specifically the cervical-thoracic boundary, which are used as start and end markers for measuring spine region lengths. On the other hand, if only taking measurements from one of the scans, the standard being the coronal scans, there is an extra level of error to consider. It would perhaps be useful to validate the method of measuring spine traits from CT scans against carcass measurements after slaughter.

Table 5.1 Correlation (r) and regression (b) coefficients between spine region length measurements[1] based on coronal and sagittal topograms within breed[2].

	Regessor	Regressand	r	L_CI	H_CI	b	L_CI	H_CI
CHA n = 51	SPL_{LUM_C}	SPL_{LUM_S}	0.493	0.252	0.677	0.347	0.171	0.523
	SPL_{THOR_C}	SPL_{THOR_S}	0.607	0.398	0.756	0.583	0.364	0.802
	SPL_{T+L_C}	SPL_{T+L_S}	0.644	0.448	0.781	0.568	0.375	0.762
SUF n = 54	SPL_{LUM_C}	SPL_{LUM_S}	0.789	0.660	0.872	0.647	0.507	0.788
	SPL_{THOR_C}	SPL_{THOR_S}	0.738	0.586	0.840	0.804	0.599	1.008
	SPL_{T+L_C}	SPL_{T+L_S}	0.863	0.774	0.918	0.846	0.708	0.983
TEX n = 50	SPL_{LUM_C}	SPL_{LUM_S}	0.614	0.405	0.762	0.688	0.431	0.944
	SPL_{THOR_C}	SPL_{THOR_S}	0.683	0.499	0.807	0.579	0.400	0.759
	SPL_{T+L_C}	SPL_{T+L_S}	0.682	0.498	0.807	0.663	0.456	0.869

r and b, correlation coefficient and regression coefficient with their 5% confidence intervals (L_CI and H_CI)

[1] SPL = length of spine region; subscript LUM, THOR and T+L defines the lumbar, thoracic and thoracolumbar spine region, respectively; subscript C and S defines the coronal and sagittal topogram

[2] CHA = Charollais; SUF = Suffolk; TEX = Texel

CT is increasingly being used to assess carcass merit of selection candidates for breeding, therefore topogram scans are readily available and spine traits can be measured. The measurements taken to describe the carcass merit can also be used to assess any phenotypic relationships between the CT-derived spine traits and CT-derived production traits. Though some crude suggestions could be extrapolated from the results obtained, little evidence was made apparent to confirm that these relationships would be consistent. This was particularly true for genetic correlations between the spine traits and production traits, the large standard errors made clear that accuracy of estimates was low.

Further to the genetic analysis component of the thesis, heritability estimates generated were somewhat unexpected. This particularly refers to the thoracic and lumbar vertebrae number traits, the heritability estimate for the thoracic vertebrae number trait, for example, was extremely high at 0.99. Further to this, vertebrae number traits were tested with a binomial distribution applied, estimates for the genetic variance of these traits are not on the scale of numbers of vertebrae, making it more challenging to interpret. As more information is added to the data sets this may reveal that a wider array of vertebrae number categories exist within the population and/or a higher frequency of animals fall into the "extreme" vertebrae number categories. This may remove the restriction of treating the vertebrae number with a binomial distribution (and combining vertebrae number categories so that data falls into either one of two definite binary groups, coded 0 or 1; Chapter 4) in future genetic analyses.

Overall, higher levels of CT scanning allowing for larger data sets to be analysed is an important future goal. Improving accuracy of heritability estimates and estimates of breeding values depend on the wide extent of information on relatives (size of pedigree) that is available to use. As the amount of information on relatives increases, and is included in the pedigree, the heritability estimate changes and the closer the calculated estimated breeding value will be to the true breeding value. Given the size of the progeny groups for sires in this study (Chapter 4) it is important to obtain more records for more accurate and reliable heritability and, subsequently, breeding value estimates.

5.1 Summary of benefits to industry

A further study (Lambe et al., 2015) expanding on that investigated and presented in this thesis included the analysis of a further data set with over 2,500 elite rams. The study showed genetic correlations which suggest that breeders may be able to select rams on spine traits, alongside growth and carcass composition traits, with no detrimental effects on current breeding goals (selection index) (Lambe et al., 2015). Further to this, the calculation of genetic parameters for these new spine traits means preliminary estimated breeding values for spine traits can be calculated for individual animals and included into selection index methods for the selection of sires and female breeding stock.

Given that genetic variation has been found in number and length of vertebrae and in spine lengths, there may be the potential to apply a selective breeding approach on sheep breeds to increase the vertebrae number or length in different spinal regions. If further studies on larger

data sets confirm this, there is the potential to produce more, or thicker, cuts from these areas of the carcass (rack of ribs, loin chops; Figure 1.10). This would provide an additional return and equate to a considerable financial gain with no increase in production or processing costs. If increased financial returns were achieved by producers then it would be predicted that there might be a higher demand for breeds of sheep selected for increased vertebral number. Market supply and demand should then provide returns for both the breeders and finishers of such lambs.

Implementing a selection strategy based on these new CT traits is not complex because these new spine traits can be measured on existing CT images. The traits could be both assessed retrospectively on existing CT images, and also introduced as routine measurements to be taken from future CT scans adding further value to the CT scanning process. Not only this, but if selection for CT-based spine traits proves to increase the proportion of saleable meat yield of high priced cuts from specific spine regions then this would also be economically beneficial in terms of the future commercial use of value-based marketing/payment systems in abattoirs (e.g. video image analysis (VIA)). VIA machines have been designed to be used on the slaughter line in abattoirs to grade/classify a carcass and predict saleable meat yield from each. Their introduction will open the opportunity of producers being paid on the basis of meat yield (HCC, 2007).

5.2 Conclusion

The present study shows that there is the potential to consider the inclusion of spine traits in a selective breeding strategy. In particular, the data suggest that selection for greater spine length/vertebrae number may be a useful consideration, particularly for the Texel (or any other) breed in which there is a higher frequency of individuals that possess fewer vertebrae. Clearly, more information is required to extend and confirm the findings of this study before such selection strategies are used in practice. However, once confirmed, implementation of additional CT derived traits would be a straightforward and relatively low cost addition to current traits used for selection. In addition, the current study has, through exploring spine traits, made an important contribution to the diversity of trait information available to breeders and the sheep industry supply chain in general.

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Appendix I Breed-specific prediction equations for predicting carcass composition of Texel lambs.

Table below (adapted from Macfarlane et al., 2006) shows the intercepts and coefficients (and SE) for equations to predict carcass fat, bone and muscle in X-ray computed tomography (CT) scans taken at the anatomical positions, ischium (ISC), 5th lumbar vertebra (LV5) and 8th thoracic vertebra (TV8), with live weight (LWT) included, for Texel (TEX) lambs; all variables as log values (adjustments to the intercepts for the groups that differ significantly are shown).

Reference	Breed/Cross	Tissue (Carcass)	Intercept	Coefficients				Adjustments to intercept[1]	R^2	r.s.d
				ISC	LV5	TV8	LW	Female		
Macfarlane et al., 2006	TEX	Fat	-6.790 (0.132)	0.073 (0.051)	0.202 (0.046)	0.388 (0.055)	0.683T (0.049)	.	0.986	0.077
		Bone	-4.690 (0.426)	0.186 (0.044)	0.170 (0.043)	0.152 (0.043)	0.499 (0.036)	-0.059 (0.015)	0.925	0.071
		Muscle	-8.087 (0.558)	0.399 (0.088)	0.284 (0.061)	0.210 (0.050)	0.493 (0.034)	0.033 (0.012)	0.966	0.052

[1] Adjustments to the intercept should be added to the general intercept for the prediction equation for female Texel lambs i.e. a female Texel lamb would have an intercept of -4.690 + -0.059 = -4.749 for the prediction of bone in the carcass

Appendix II Estimates of phenotypic (above diagonal) and genetic (*below diagonal*) correlation coefficients (and SE) between CT-derived traits and heritability estimates[1] (**on diagonal**) (and SE).

Trait	CTwt	LD_W	LD_D	LD_A	Pfat	Pmusc	Pbone	Ptotal	Pfat(%)	Pmusc(%)	Pbone(%)
CTwt	**0.65 (0.260)**	0.786 (0.022)	0.734 (0.027)	0.807 (0.023)	0.934 (0.008)	0.953 (0.006)	0.923 (0.009)	0.983 (0.002)	0.871 (0.015)	-0.753 (0.027)	-0.815 (0.020)
LD_W	*0.850 (0.112)*	**0.32 (0.179)**	0.231 (0.052)	0.604 (0.036)	-0.148 (0.054)	0.465 (0.043)	0.271 (0.048)	0.305 (0.047)	-0.079 (0.057)	0.267 (0.051)	-0.262 (0.049)
LD_D	*0.827 (0.126)*	*0.840 (0.214)*	**0.30 (0.180)**	0.809 (0.018)	0.035 (0.055)	0.531 (0.041)	0.056 (0.053)	0.493 (0.039)	0.049 (0.057)	0.206 (0.055)	-0.397 (0.046)
LD_A	*0.744 (0.153)*	*0.893 (0.107)*	*0.985 (0.054)*	**0.69 (0.276)**	0.005 (0.062)	0.631 (0.041)	0.132 (0.056)	0.562 (0.038)	0.010 (0.066)	0.258 (0.061)	-0.407 (0.050)
Pfat	*0.967 (0.024)*	*-0.255 (0.379)*	*0.192 (0.396)*	*0.131 (0.351)*	**0.42 (0.204)**	-0.289 (0.052)	-0.336 (0.046)	0.398 (0.049)	0.928 (0.008)	-0.841 (0.016)	-0.378 (0.046)
Pmusc	*0.961 (0.026)*	*0.750 (0.200)*	*0.380 (0.325)*	*0.524 (0.244)*	*-0.737 (0.211)*	**0.50 (0.218)**	0.224 (0.050)	0.704 (0.026)	-0.281 (0.054)	0.633 (0.033)	-0.444 (0.048)
Pbone	*0.972 (0.024)*	*0.414 (0.385)*	*0.500 (0.400)*	*0.292 (0.389)*	*-0.697 (0.289)*	*0.600 (0.303)*	**0.21 (0.144)**	0.133 (0.050)	-0.267 (0.049)	0.078 (0.053)	0.351 (0.045)
Ptotal	*0.994 (0.005)*	*0.756 (0.248)*	*0.653 (0.265)*	*0.773 (0.174)*	*-0.266 (0.436)*	*0.823 (0.151)*	*0.379 (0.424)*	**0.25 (0.149)**	0.274 (0.054)	-0.015 (0.054)	-0.454 (0.042)
Pfat(%)	*0.930 (0.047)*	*-0.193 (0.374)*	*0.162 (0.381)*	*0.072 (0.339)*	*0.947 (0.041)*	*-0.708 (0.215)*	*-0.520 (0.350)*	*-0.357 (0.402)*	**0.56 (0.238)**	-0.821 (0.018)	-0.534 (0.039)
Pmusc(%)	*-0.877 (0.082)*	*0.412 (0.338)*	*-0.138 (0.391)*	*0.058 (0.345)*	*-0.928 (0.060)*	*0.886 (0.110)*	*0.481 (0.372)*	*0.512 (0.363)*	*-0.928 (0.059)*	**0.47 (0.213)**	-0.045 (0.056)
Pbone(%)	*-0.929 (0.053)*	*-0.281 (0.401)*	*-0.059 (0.448)*	*-0.265 (0.362)*	*-0.541 (0.302)*	*0.040 (0.398)*	*0.338 (0.428)*	*-0.081 (0.440)*	*-0.673 (0.225)*	*0.348 (0.364)*	**0.26 (0.158)**

[1] Heritability estimates taken from the univariate analysis

Appendix II continued. Estimates of phenotypic correlation coefficients (and SE) between CT-derived traits.

Trait	KO(%)	SMY(%)	SPL_{THOR}	SPL_{LUM}	SPL_{T+L}	VL_{THOR}	VL_{LUM}	VL_{T+L}	VN_{THOR}	VN_{LUM}	VN_{T+L}
CTwt	0.822 (0.018)	0.454 (0.046)	0.729 (0.028)	0.476 (0.040)	0.823 (0.018)	0.749 (0.028)	0.806 (0.020)	0.826 (0.020)	-0.039 (0.079)	-0.043 (0.039)	-0.057 (0.058)
LD_W	0.420 (0.042)	0.503 (0.039)	0.115 (0.053)	0.101 (0.051)	0.193 (0.048)	0.040 (0.058)	0.082 (0.049)	0.066 (0.057)	0.091 (0.059)	-0.032 (0.033)	0.033 (0.047)
LD_D	0.538 (0.036)	0.552 (0.039)	0.017 (0.054)	-0.158 (0.049)	-0.119 (0.050)	-0.020 (0.057)	-0.079 (0.050)	-0.076 (0.055)	0.015 (0.066)	-0.078 (0.034)	-0.051 (0.049)
LD_A	0.603 (0.034)	0.633 (0.038)	0.076 (0.058)	-0.108 (0.053)	-0.020 (0.054)	-0.042 (0.065)	-0.003 (0.054)	-0.074 (0.062)	0.077 (0.068)	-0.075 (0.035)	-0.009 (0.052)
Pfat	0.341 (0.047)	-0.220 (0.052)	-0.094 (0.055)	-0.023 (0.052)	-0.103 (0.051)	-0.108 (0.059)	-0.063 (0.050)	-0.116 (0.057)	Not estimable[1]	Not estimable[1]	Not estimable[1]
Pmusc	0.730 (0.026)	0.953 (0.005)	0.098 (0.056)	-0.150 (0.050)	-0.043 (0.052)	0.029 (0.061)	-0.055 (0.051)	-0.029 (0.059)	-0.006 (0.073)	-0.071 (0.036)	-0.051 (0.055)
Pbone	0.214 (0.048)	0.241 (0.049)	0.198 (0.050)	-0.001 (0.049)	0.151 (0.049)	0.206 (0.053)	0.118 (0.048)	0.198 (0.052)	Not estimable[1]	Not estimable[1]	Not estimable[1]
Ptotal	0.874 (0.013)	0.671 (0.028)	0.019 (0.052)	-0.175 (0.048)	-0.135 (0.049)	-0.048 (0.055)	-0.136 (0.048)	-0.122 (0.053)	-0.022 (0.076)	-0.051 (0.038)	-0.052 (0.057)
Pfat(%)	0.397 (0.046)	-0.132 (0.056)	-0.028 (0.057)	0.002 (0.053)	-0.023 (0.053)	-0.039 (0.062)	0.020 (0.051)	-0.030 (0.060)	-0.034 (0.085)	-0.011 (0.044)	-0.059 (0.064)
Pmusc(%)	0.008 (0.052)	0.569 (0.037)	0.066 (0.056)	-0.038 (0.051)	0.025 (0.052)	0.047 (0.060)	-0.003 (0.050)	0.031 (0.058)	0.042 (0.081)	-0.010 (0.041)	0.040 (0.060)
Pbone(%)	-0.704 (0.026)	-0.609 (0.036)	-0.053 (0.052)	0.055 (0.050)	0.021 (0.050)	-0.005 (0.057)	Not estimable[1]	0.003 (0.056)	0.012 (0.077)	0.042 (0.040)	0.071 (0.060)

[1] Model could not converge, parameters not estimable

Appendix II continued. Estimates of genetic correlation coefficients (and SE) between CT-derived traits.

Trait	CTwt	LD_W	LD_D	LD_A	Pfat	Pmusc	Pbone	Ptotal	Pfat(%)	Pmusc(%)	Pbone(%)
KO(%)	*0.939* *(0.053)*	*0.943* *(0.182)*	*0.700* *(0.271)*	*0.862* *(0.168)*	*-0.035* *(0.464)*	*0.622* *(0.260)*	*0.378* *(0.451)*	*0.732* *(0.206)*	*0.101* *(0.432)*	*0.187* *(0.441)*	*-0.576* *(0.312)*
SMY(%)	*0.443* *(0.300)*	*0.860* *(0.165)*	*0.362* *(0.353)*	*0.564* *(0.251)*	*-0.614* *(0.282)*	*0.956* *(0.035)*	*0.570* *(0.326)*	*0.790* *(0.181)*	*-0.506* *(0.314)*	*0.751* *(0.195)*	*-0.203* *(0.396)*
SPL_{THOR}	*0.852* *(0.103)*	*0.750* *(0.301)*	*0.558* *(0.384)*	*0.530* *(0.325)*	*-0.554* *(0.313)*	*0.764* *(0.236)*	*0.403* *(0.390)*	*0.420* *(0.398)*	*-0.311* *(0.359)*	*0.653* *(0.291)*	*-0.575* *(0.376)*
SPL_{LUM}	*0.813* *(0.204)*	*-0.513* *(0.476)*	*-0.598* *(0.458)*	*-0.460* *(0.444)*	*0.937* *(0.299)*	*-0.762* *(0.316)*	*-0.236* *(1.406)*	*-0.370* *(0.524)*	*0.893* *(0.341)*	*-0.797* *(0.356)*	*-0.682* *(0.587)*
SPL_{T+L}	*0.953* *(0.043)*	*0.593* *(0.446)*	*0.280* *(0.548)*	*0.420* *(0.461)*	*0.063* *(0.480)*	*0.361* *(0.438)*	*-0.607* *(0.568)*	*0.273* *(0.525)*	*0.247* *(0.445)*	*0.217* *(0.454)*	*-0.625* *(0.588)*
VL_{THOR}	*0.844* *(0.099)*	*-0.202* *(0.368)*	*0.191* *(0.400)*	*-0.055* *(0.344)*	*-0.244* *(0.344)*	*0.284* *(0.330)*	*-0.003* *(0.430)*	*-0.046* *(0.397)*	*0.010* *(0.348)*	*0.288* *(0.334)*	*-0.678* *(0.305)*
VL_{LUM}	*-0.113* *(3.109)*	*0.993* *(0.525)*	*0.785* *(0.539)*	*0.869* *(0.410)*	*-0.019* *(0.561)*	*0.488* *(0.501)*	*-0.438* *(0.715)*	*0.420* *(0.619)*	*0.147* *(0.542)*	*0.309* *(0.521)*	*Not estimable*[1]
VL_{T+L}	*0.898* *(0.067)*	*-0.202* *(0.377)*	*0.174* *(0.417)*	*-0.061* *(0.356)*	*-0.124* *(0.368)*	*0.206* *(0.351)*	*-0.230* *(0.455)*	*-0.076* *(0.407)*	*0.119* *(0.356)*	*0.217* *(0.352)*	*-0.866* *(0.259)*
VN_{THOR}	*-0.424* *(0.323)*	*0.273* *(0.391)*	*-0.097* *(0.400)*	*0.116* *(0.390)*	*Not estimable*[1]	*-0.283* *(0.357)*	*Not estimable*[1]	*-0.353* *(0.341)*	*-0.396* *(0.320)*	*0.376* *(0.324)*	*0.391* *(0.336)*
VN_{LUM}	*-0.042* *(0.654)*	*-0.807* *(0.652)*	*-0.632* *(0.660)*	*-0.646* *(0.667)*	*Not estimable*[1]	*-0.507* *(0.685)*	*Not estimable*[1]	*-0.117* *(0.669)*	*0.447* *(0.587)*	*-0.565* *(0.552)*	*-0.127* *(0.669)*
VN_{T+L}	*-0.327* *(0.387)*	*0.127* *(0.467)*	*-0.372* *(0.411)*	*-0.078* *(0.452)*	*Not estimable*[1]	*-0.312* *(0.398)*	*Not estimable*[1]	*-0.281* *(0.401)*	*-0.268* *(0.388)*	*0.148* *(0.399)*	*0.431* *(0.374)*

[1] Model could not converge, parameters not estimable

Appendix II continued. Estimates of phenotypic (above diagonal) and genetic (*below diagonal*) correlation coefficients (and SE) between CT-derived traits and heritability estimates[1] (**on diagonal**) (and SE).

Trait	KO(%)	SMY(%)	SPL_{THOR}	SPL_{LUM}	SPL_{T+L}	VL_{THOR}	VL_{LUM}	VL_{T+L}	VN_{THOR}	VN_{LUM}	VN_{T+L}
KO(%)	**0.18 (0.132)**	0.820 (0.017)	0.106 (0.051)	-0.144 (0.048)	-0.030 (0.050)	0.037 (0.054)	Not estimable[2]	-0.009 (0.053)	0.031 (0.069)	-0.071 (0.036)	-0.047 (0.054)
SMY(%)	*0.785 (0.179)*	**0.34 (0.175)**	0.133 (0.054)	-0.135 (0.049)	0.003 (0.051)	0.068 (0.058)	0.003 (0.050)	0.023 (0.056)	0.072 (0.056)	-0.057 (0.023)	-0.026 (0.043)
SPL_{THOR}	*0.931 (0.280)*	*0.980 (0.175)*	**0.30 (0.172)**	-0.374 (0.042)	0.600 (0.032)	0.811 (0.021)	0.395 (0.041)	0.689 (0.029)	0.127 (0.072)	-0.233 (0.033)	-0.080 (0.053)
SPL_{LUM}	*-0.232 (0.619)*	*-0.676 (0.379)*	*-0.650 (0.373)*	**0.08 (0.095)**	0.517 (0.037)	-0.126 (0.050)	-0.009 (0.048)	0.107 (0.050)	-0.195 (0.046)	0.393 (0.021)	0.216 (0.040)
SPL_{T+L}	*0.959 (0.379)*	*0.730 (0.380)*	*0.853 (0.189)*	*-0.158 (0.665)*	**0.14 (0.110)**	0.642 (0.031)	0.342 (0.044)	0.730 (0.024)	-0.014 (0.070)	0.045 (0.036)	0.051 (0.057)
VL_{THOR}	*0.431 (0.398)*	*0.480 (0.318)*	*0.720 (0.180)*	*-0.418 (0.475)*	*0.713 (0.247)*	**0.55 (0.241)**	0.393 (0.043)	0.937 (0.007)	-0.129 (0.080)	-0.131 (0.041)	-0.207 (0.062)
VL_{LUM}	*Not estimable[2]*	*0.846 (0.464)*	*0.887 (0.351)*	*0.290 (0.853)*	*0.623 (0.955)*	*0.608 (0.451)*	**0.08 (0.086)**	0.614 (0.032)	0.009 (0.064)	-0.152 (0.032)	-0.127 (0.045)
VL_{T+L}	*0.476 (0.409)*	*0.441 (0.340)*	*0.684 (0.213)*	*-0.253 (0.529)*	*0.765 (0.217)*	*0.988 (0.014)*	*0.669 (0.394)*	**0.44 (0.211)**	-0.135 (0.077)	-0.080 (0.041)	-0.174 (0.061)
VN_{THOR}	*-0.146 (0.379)*	*0.237 (0.397)*	*-0.227 (0.382)*	*-0.419 (0.412)*	*-0.340 (0.361)*	*-0.627 (0.265)*	*-0.380 (0.378)*	*-0.615 (0.270)*	**0.99 (0.419)**	NA	NA
VN_{LUM}	*-0.338 (0.693)*	*-0.261 (2.340)*	*-0.681 (0.604)*	*0.831 (0.336)*	*-0.193 (0.719)*	*-0.326 (0.663)*	*0.091 (0.740)*	*-0.133 (0.672)*	*NA*	**0.08 (0.125)**	NA
VN_{T+L}	*-0.224 (0.429)*	*-0.149 (0.457)*	*-0.346 (0.404)*	*-0.282 (0.519)*	*-0.429 (0.406)*	*-0.864 (0.191)*	*-0.201 (0.453)*	*-0.771 (0.240)*	*NA*	*NA*	**0.44 (0.275)**

[1] Heritability estimates taken from the univariate analysis
[2] Model could not converge, parameters not estimable