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Citation for published version:
Clelland, N, Bunger, L, McLean, KA, Knott, S, Matthews, KR & Lambe, NR 2018, 'Prediction of intramuscular fat content and shear force in Texel lamb loins using combinations of different X-ray computed tomography (CT) scanning techniques' Meat Science, vol. 140, pp. 78-85. DOI: 10.1016/j.meatsci.2018.03.003

Digital Object Identifier (DOI):
10.1016/j.meatsci.2018.03.003

Published In:
Meat Science

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

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Abstract

Computed tomography (CT) parameters, including spiral computed tomography scanning (SCTS) parameters, intramuscular fat (IMF) and mechanically measured shear force were derived from two previously published studies. Purebred Texel (n = 377) of both sexes, females (n = 206) and intact males (n = 171) were used to investigate the prediction of IMF and shear force in the loin. Two and three dimensional CT density information was available. Accuracies in the prediction of shear force and IMF ranged from R^2 0.02 to R^2 0.13 and R^2 0.51 to R^2 0.71 respectively, using combinations of SCTS and CT scan information. The prediction of mechanical shear force could not be achieved at an acceptable level of accuracy employing SCTS information. However, the prediction of IMF in the loin employing information from SCTS and additional information from standard CT scans was successful, providing evidence that the prediction of IMF and related meat eating quality (MEQ) traits for Texel lambs *in vivo* can be achieved.

Keywords: spiral x-ray computed tomography, lamb, meat quality, intramuscular fat.

1. Introduction

Computed tomography (CT) is a non-invasive, diagnostic tool initially developed for use in human medicine to improve the imaging of soft tissue structures and assist in diagnosing conditions or diseases not directly associated with bone structure. Over the last few decades CT has been adopted for use in animal breeding and is now routinely used in selective breeding programs for sheep in the UK to accurately estimate carcass composition of live animals. More recently, the prediction of aspects of meat quality (MQ) such as intramuscular fat levels (IMF), fatty acid profiles and tissue composition have been investigated both *in vivo* and post mortem in meat producing species (Font-i-Furnols, Brun, Tous, & Gispert, 2013; Kongsro & Gjerlaug-enger, 2013; L. Bünger, J.M. Macfarlane, N. R. Lambe, J. Conington, K. A. McLean, K. Moore, 2011; Prieto et al., 2010). The basic principle of CT is the measurement of the spatial distribution of any physical quantity. Offering greater contrast in the imaging of soft tissue to that seen in conventional radiography (Kalender, 2006). The first method of image capture most commonly used is 'single-slice' scanning. During single-slice scanning, x-rays are used to generate cross-sectional, two-dimensional images of the selected region of a subject. Each image is produced by rotation of the x-ray tube 360^{o} around the subject. Attenuation of radiation through the tissues can then be measured, with differences indicating different tissue densities.

Advances in scanning technology have resulted in the development of contiguous scanning procedures such as spiral CT scanning (SCTS), capable of producing a series of images in a single contiguous scan at intervals of as little as 0.6 mm apart. The advantage is that multiple images can be acquired faster, at reduced intervals, resulting in increased information acquisitions in less time. Recent studies provide evidence that muscle density information from single or multiple CT scans in sheep, can provide moderately accurate predictions of IMF content *in vivo*. Prediction accuracies range from R^2 = 0.33 to 0.68 using several approaches including various CT parameters (Clelland et al., 2014; Karamichou, Richardson, Nute, McLean, & Bishop, 2006; Lambe et al., 2008; Lambe, McLean, et al., 2010; J. M. Macfarlane, 2006). The aim of this study was to investigate any gains in the prediction of IMF content and shear force in the loins of Texel sheep that may be achieved by utilizing the wealth of information that relatively new SCTS techniques may provide.

2. Materials and Methods

2.1. Experimental animals

The CT parameters, including SCTS parameters, IMF and shear force in the loin were derived from two previously-published studies. The first of these studies (Exp. 1), was conducted over two years (2003 to 2004) and investigated the use of various *in vivo* measurement techniques (ultrasonography, video image analysis and CT), to predict carcass and meat quality in purebred Texel (n = 240) and Scottish Blackface (n = 233) lambs. The full study and methods are detailed in Lambe et al. (2008). The second study (Exp. 2) was conducted in 2009 and investigated the genotypic effects of the Texel muscling quantitative trait loci (TMQTL) on carcass and meat quality in purebred Texel lambs (n = 137). Full details are published in Lambe et al. (2010). The combination of these data from Exp. 1 and Exp. 2 comprised data from pure-bred Texel lambs (n = 377) of both sexes, females (n = 206) and intact males (n = 171). Lambs were reared to weaning as either singles (n = 184), twins (n = 168) or artificially hand reared (n = 25). Mean age at CT was 132 d (SD 21.1, range 91-202 d); with mean live weight 35.3kg (SD 4.9, range 20-49kg). Lambs were CT scanned pre-slaughter using a Siemens Somatom Esprit scanner. All lambs were lightly sedated (Rompun®, Bayer animal health, Bayer plc., Newbury, UK) at a dose of 0.1-0.2mg xylazine hydrochloride/kg body weight, then secured in a purpose-built cradle before being CT-scanned.

2.2. Single-slice and spiral x-ray CT measurements and image analysis

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

Insert Figure 1 Here

This two dimensional method of scanning at these particular anatomical sites (including an additional scan at the 5th lumbar vertebra, which was not used in this study), is currently used in UK terminal sire breeding programs to provide accurate predictions of fat and muscle weights in the carcass. This method, defined as ‘reference’ scanning (L. Bünger, J.M. Macfarlane, N. R. Lambe, J. Conington, K. A. McLean, K. Moore, 2011), optimizes the number of images required to be taken across the body of the sheep while maximizing the accuracy of estimations for carcass traits. Images were produced with a resolution of 512 x 512 pixels with a 450mm field of view, producing images with a pixel size of $0.77mm^2$ in two dimensions. Spiral images were produced at the same resolution and field of view at intervals of 8mm, producing images with a voxel size of $6.2mm^3$.

Automated analyses were performed on the images produced, to separate carcass from non-carcass tissues (Glasbey & Young, 2002), and calculate the density of each pixel in Hounsfield units (HU), the standard quantitative scale for describing radiodensity. In the final segmented image each pixel was allocated to fat, muscle or bone using image thresholding techniques (Mann, Young, Glasbey, & McLean, 2003). The thresholds in Hounsfield units (HU) defined for the CT scanner (Siemens Somatom Esprit single slice) were Fat = -174 to -12HU, Muscle = -10 to 92HU and Bone = 94HU and above, based on previous calibration trials. Areas (mm^2) and average densities (HU) of muscle and fat in each two dimensional image were calculated, as well as standard deviations of the density values allocated to each tissue. Combining all pixels allocated as either fat or muscle enabled the use of a novel average 'soft tissue density' and standard deviation. The SCTS images were used to calculate weighted average densities of muscle, fat and soft tissue (average tissue density, in each individual scan image, weighted for tissue area in that image and averaged across all images in the spiral scan series). Volumes of each tissue (mm^3) were also calculated. The resulting SCTS parameters included; weighted muscle and fat densities and relating standard deviations, weighted soft tissue densities and standard deviation, and calculated muscle and fat volumes (mm^3). The CT parameters measured from the two dimensional reference scans in the ISC and TV8 regions were muscle density, fat density and related standard deviations, as well as the soft tissue densities and standard deviations of soft tissue densities. Muscle area and fat area tissue measurements (mm^2) were also calculated for each of the reference scan images. Total CT predicted carcass fat (PrCfat), as a measure of subcutaneous and intermuscular fat in the entire carcass, was also predicted using a breed-specific prediction equation developed from previous research (Macfarlane et al., 2006):

$$PrCfat(kg) = (-2236 + (LW \times 80.26) + (ISCFA \times 0.21) + (LV5FA \times 0.19) + (TV8FA \times 0.221))/1000$$

Where PrCfat is the CT predicted weight of subcutaneous and intermuscular fat (kg), LW is live weight at CT scanning, ISCFA is the area of pixels allocated as fat in the scan image taken at the ischium (mm^2), LV5FA is the area of pixels allocated as fat in the scan image taken at the 5th lumbar vertebra (mm^2) and TV8FA is the area of pixels allocated as fat in the scan image taken at the 8th thoracic vertebra. Details, acronyms and descriptions of each CT and MQ trait are presented in Table1.

2.3. Slaughter procedure and meat quality parameter measurements

The loin muscle (*M. longissimus lumborum*) was removed from the right side of each carcass included in Exp. 1, vacuum-packed aged for 7 days, and frozen prior to meat quality analysis at the University of Bristol. Carcasses included in Exp. 2 were subjected to high voltage electrical stimulation (700 volts RMS for 45 seconds applied between the end of the processing line and the chiller), chilled and aged for between 7-9 d and dissected, removing the loin muscle (*M. longissimus lumborum*) from the right side of the carcass. In both Exp. 1 and 2, IMF content was measured in a cross-sectional slice taken from the cranial end of the muscle at the first lumbar vertebra. Each sample was blended to a fine paste and IMF content was measured using petroleum ether (B.P. 40-60^{o}C) as the solvent in a modified Soxhlet extraction (AOAC, 1990). Mean IMF was 1.48% (SD 0.68) and ranged from 0.27 – 3.88%. The majority of lambs were slaughtered 4-8 d after CT scanning (n=217), and the remaining lambs were slaughtered 32-33 d after CT scanning (n=160), to allow for a 30 day withdrawal period from the CT sedative and subsequent taste panel analysis (which formed part of a wider study). Shear force was also measured, using a TA-XT2 texture analyzer (Stable Micro System, Surrey, UK) fitted with Volodkevich-type jaws, a standard compression method to determine tenderness simulating the action of the incisor tooth (Volodkevich, 1938). Loins were cooked 'sous-vide' (in vacuum pack bags) in a water bath at 80^{o}C to an internal core temperature of 78^{o}C (Teye et al., 2006) monitoring individual loin temperature using a digital temperature probe (Hanna Instruments UK, Eden Way, Bedfordshire) . Samples were then immediately cooled in iced water and held at 4^{o}C overnight for a minimum period of 12 hours. Ten 10 x 10 x 20mm samples were cut from each loin following the direction of the muscle fibers and sheared at a constant speed of 1mm/s perpendicular to the muscle fiber direction. Shear force was recorded as the force required (kgF) to compress the sample, with greater values for less tender samples. Results were averaged over the ten samples taken from each loin. Mean shear force was 3.4kgF (SD 1.56) and ranged from 1.39 – 10.72kgF.

2.4. Statistical analysis

Lambs with no IMF data were removed (n = 2), lambs without full CT information were removed (n = 2), and finally lambs with IMF content greater than three standard deviations from the mean were identified as outliers and also removed (n = 3). Initial regression analysis and subsequent model checking (Distribution of residuals) suggested the need for transformation of shear force data. As a result shear force was log-transformed and fitted to a normal distribution prior to any regression analysis. The number of days from CT scanning to slaughter (group 1: 4-8 d; group 2: 32-33 d, accounting for lambs subjected to a withdrawal period to allow subsequent taste panel analysis) was tested using a general analysis of variance in Genstat14™ adjusted for PrCfat, and provided evidence of no significant effect on IMF content (P = 0.80) or shear force (P = 0.07). The term was also fitted as an independent variable in the simple regression models in order to test the relationship between days to slaughter and the CT parameters and was again not significant when tested on IMF (P = 0.71) and shear force (P = 0.19) therefore was not included in the analysis. A summary of the CT traits tested in the models are presented in Table 1. Histograms of MQ traits (shear force prior to transformation and IMF) are presented in Fig. 2.

Table 1: Acronyms and summary statistics of both CT and meat quality traits along with trait descriptions, means and standard deviations (SD) in the Texel data utilized in the prediction of IMF (n = 370)

Trait	Acronym	Trait Description	Mean	SD
CT Traits				
	ISCMD	Average muscle density in 2D scan at the ischium (HU)	48.44	2.10
	ISCMSD	SD of muscle density in 2D scan at the ischium (HU)	16.81	0.81
	ISCFD	Average fat density in 2D scan at the ischium (HU)	-62.37	5.32
	ISCFSD	SD of fat density in 2D scan at the ischium (HU)	36.51	2.50
	ISCFA	Carcass fat area measured in 2D scan at the ischium (mm^2)	3651	1404
	ISCMA	Muscle area measured in 2D scan at the ischium (mm^2)	27415	2898
	TV8MD	Average muscle density in 2D scan at the 8^{th} thoracic vertebra (HU)	44.68	2.98
	TV8MSD	SD of muscle density in 2D scan at the 8^{th} thoracic vertebra (HU)	21.94	1.73
	TV8FD	Average fat density in 2D scan at the 8^{th} thoracic vertebra (HU)	-64.64	5.99
	TV8FSD	SD of fat density in 2D scan at the 8^{th} thoracic vertebra (HU)	39.21	3.16
	TV8FA	Carcass fat area measured in 2D scan at the 8^{th} thoracic vertebra (mm^2)	3451	1843
	TV8MA	Muscle area measured in 2D scan at the 8^{th} thoracic vertebra (mm^2)	12380	1833
	ISCSTD	Average soft tissue density in 2D scan at the ischium (HU)	35.55	5.07
	ISCSTSD	SD of soft tissue density in 2D scan at the ischium (HU)	40.34	5.66
	TV8STD	Average soft tissue density in2D scan at the 8^{th} thoracic vertebra (HU)	21.84	11.35
	TV8STSD	SD of soft tissue density in 2D scan at the 8^{th} thoracic vertebra (HU)	50.56	6.69
	w_md	Average muscle density in the loin spiral scan (weighted by area in each component image) (HU)	46.13	2.22
	w_msd	SD of muscle density in the loin spiral scan (weighted by area in each component image) (HU)	19.91	1.25
	w_fd	Average fat density in the loin spiral scan (weighted by area in each component image) (HU)	-63.97	4.65
	w_fsd	SD of fat density in the loin spiral scan (weighted by area in each component image) (HU)	40.63	3.49
	m_vol	Muscle tissue volume in the loin spiral scan (cm^3)	1827	281
	f_vol	Fat tissue volume in the loin spiral scan (cm^3)	298	180
	w_std	Soft tissue density in the loin spiral scan weighted by area (HU)	31.41	8.43
	w_stsd	SD of soft tissue in the loin spiral scan weighted by area (HU)	42.79	6.17
	PrCfat	Predicted total carcass fat weight (kg)	2.34	1.11
MQ Traits				
	Shear force	*M. longissimus lumborum* shear force (kgF)	3.40	1.56
	IMF	*M. longissimus lumborum* intra-muscular fat (%)	1.48	0.68

Insert Figure 2 Here

Sixteen models were tested in the analyses (Table 2), termed models A-P using information from SCTS only (^{sp}) and a combination of SCTS and reference information (^{com}). Models with one or two variables included in the maximum model were analyzed using simple and multiple linear regression, respectively, whilst models employing CT data with more than two variables were analyzed using stepwise linear regression in Genstat14™ (Payne, Murray, Harding, Baird, & Soutar, 2011), to optimize the number and combination of independent variables from the maximum fitted model. Models were then tested for significant differences between correlation coefficients ($\sqrt{\text{Adj } R^2}$) applying standard methods using Fisher's Z transformation (Mudholkar, 2006). Final models were identified as those with significantly greater prediction accuracies of MQ traits than the baseline model (Model A). These models were then validated. During validation, available data were split using a natural time series separation in the data, as described by Snee (1977). Experiment one data was employed as a calibration data set, and experiment two data as a validation data set. Summary statistics for MQ traits and CT measured traits for both calibration and validation data sets are presented in Table 3, Histograms of MQ traits (shear force prior to transformation and IMF) are presented in Fig. 3.

Table 2: Terms included in the maximum linear regression models tested prior to stepwise regression using both spiral CT scan parameters only (sp) and spiral CT scan parameters alongside two-dimensional reference scan parameters (com). Explanations of acronyms used in the models can be found in Table 1

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

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

Insert Figure 3 Here

Table 3: Acronyms and summary statistics of both CT and meat quality traits, means and standard deviations (SD) in the calibration and validation data sets: trait descriptions, means and standard deviations (SD)

		Calibration Data (n=236)		Validation Data (n=134)	
Trait	Acronym	Mean	SD	Mean	SD
CT Traits					
	ISCMD	49.32	1.78	46.90	1.69
	ISCMSD	16.87	0.76	16.71	0.89
	ISCFD	-63.48	5.59	-60.43	4.14
	ISCFSD	35.89	1.97	37.57	2.97
	ISCFA	3999	1425	3060	1164
	ISCMA	28328	2486	25823	2887
	TV8MD	44.89	2.98	44.24	2.97
	TV8MSD	21.52	1.68	22.69	1.56
	TV8FD	-64.66	6.58	-64.59	4.84
	TV8FSD	38.48	2.93	40.45	3.18
	TV8FA	3603	1990	3209	1541
	TV8MA	12859	1646	11533	1834
	ISCSTD	35.39	5.45	35.77	4.33
	ISCSTSD	41.77	5.88	37.88	4.23
	TV8STD	21.87	12.25	21.62	9.66
	TV8STSD	50.32	7.41	51.06	5.16
	PrCfat	2.60	1.08	1.88	1.01
	w_md	45.98	2.30	46.40	2.05
	w_msd	20.11	1.25	19.55	1.19
	w_fd	-64.36	4.41	-63.29	5.01
	w_fsd	40.37	3.46	41.08	3.52
	m_vol	1908	261	1686	259
	f_vol	329	195	244	133
	w_std	30.35	9.18	33.27	6.55
	w_stsd	43.65	6.57	41.27	5.09
MQ Traits					
	Shear force	3.73	1.69	2.82	1.08
	IMF	1.60	0.79	1.31	0.54

3. Results

3.1. Predicting shear force and IMF content using SCTS information

Very little of the variation in shear force was accounted for by PrCfat (Adj $R^2 = 0.05$), however PrCfat accounted for a moderate amount of the variation in IMF (Adj $R^2 = 0.50$). Compared to the baseline (Model A; Table 2), using only information from CT derived predicted carcass fat, seven models that included additional CT variables, from the fifteen models tested, were identified as being statistically significantly more accurate in the prediction of IMF ($P > 0.05$). None of the additive models using only spiral CT information were significantly more accurate ($P < 0.05$) in prediction of shear force when compared to the baseline (Table 4).

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

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

The six remaining models including only SCTS information identified as better predictors of IMF than PrCfat alone were compared with the maximum benchmark (Model L). Models with significantly reduced accuracy ($P > 0.05$) compared to the benchmark model L were discarded. This included model P (Table 4), which left a total of six models with correlation coefficients that were not significantly different, essentially meaning that the prediction ability of these six models is statistically similar, thus identifying a group of models that would predict IMF equally using SCTS information. Model K was also dropped as it was entirely the same final model as model J following stepwise linear regression. The final selected models included; model B (Adj R^2 = 0.67), model F (Adj R^2 = 0.68), model H (Adj R^2 = 0.67), model J (Adj R^2 = 0.69) and model L (Adj R^2 = 0.70).

3.2. Predicting shear force and IMF content using a combination of SCTS and reference scan information

Models using both SCTS information (^{sp}) and a combination of SCTS information and reference information (^{com}) were again compared to the simple linear model using only PrCfat for the predictions of both shear force and IMF. In the analysis for the prediction of shear force, prediction accuracies were significantly improved with the inclusion of information from the reference scan images (ISC, TV8). Nonetheless, the overall results show that the maximum prediction accuracy achieved for shear force, from models developed was Adj R^2 = 0.13 (Table 4).

In the prediction of IMF ten of the fifteen models tested were significantly greater in prediction accuracies than that of PrCfat alone ($P < 0.05$). From these models the single ‘best’ model was identified as model L^{com} (Adj R^2 = 0.71) and used as a maximum benchmark model:

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

Where PrCfat is CT predicted carcass fat, w_md is weighted muscle density in the spiral information, m_vol is the volume of muscle estimated from the spiral information, f_vol is the volume of fat estimated from spiral information, ISCMD is the average muscle density in the ischium scan region, ISCMSD is the standard deviation of muscle density in the ischium scan region, ISCMA is the estimated area of muscle in the ischium scan region, TV8MD is the average density of muscle within the 8th thoracic vertebra region, TV8MA is the estimated muscle area within the 8th thoracic vertebra region and TV8FA is the estimated fat area within the 8th thoracic vertebra region.

All models were then tested against the benchmark and any that were statistically significantly different in prediction accuracy were discarded ($P > 0.05$), which included Model M^{com} (Adj $R^2 = 0.63$). These analyses therefore identified nine "best" models with similar prediction abilities: L^{com} (benchmark; Adj $R^2 = 0.71$); F^{com}, J^{com} and K^{com} (Adj R^2 = 0.70); B^{com} and H^{com} (Adj R^2 = 0.68); O^{com} and P^{com} (Adj R^2 = 0.67); and N^{com} (Adj R^2 = 0.66). Regression results for all models are presented in Table 4.

Table 4: Regression results for the prediction of ShF or IMF, presented is the adjusted coefficient of determination (Adj R^2) and residual mean square error (RMSE) using information from SCTS only (sp) or a combination of SCTS and two-dimensional reference scans (com), using the whole dataset (n=370).

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

^{sp} Using SCTS information

^{com} Using a combination of SCTS and reference CT information

*Adj R^2 differs significantly from the baseline model (A) ($P > 0.05$)

**Adj R^2 does not differ significantly from the maximum benchmark model ($P < 0.05$)

3.3. Model Validation and selection

Given the poor prediction abilities of CT for shear force ($R^2 < 0.30$) using the parameters tested, validation analysis for the prediction of shear force was not carried out. Fourteen possible models in the prediction of IMF were identified. None of these models had significantly less prediction accuracy ($P < 0.05$) than the single 'best' model from both SCTS information and a combination of SCTS information and reference information (Model L^{com}), so all were retained for validation analyses, with Adj R^2 ranging from 0.67 to 0.71. For validation, fourteen prediction equations were derived using the calibration data set (n = 236), corresponding to the independent variables identified in the final selected models from the primary stepwise regression analysis. The models were then used to predict the chemical IMF values of lambs included in the independent validation data set (n = 134). Final validation results, coefficients of determination (R^2) and residual mean square errors of prediction (RMSEP) are presented in Table 5.

Table5: Validation results: adjusted coefficient of determination (Adj R^2), residual mean square error (RMSE) of calibration; and coefficient of determination (R^2) and residual mean square error of prediction (RMSEP) of the validation data

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

^{sp} Model uses information from spiral scans only

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

The model with the strongest validity was model N^{com} (R^2 = 0.67, RMSEP = 0.31) using both SCTS information and reference scan information, including CT predicted carcass fat (PrCfat), weighted density of soft tissue and its standard deviation (w_std and w_stsd) in the spiral scan of the loin, soft tissue density and its standard deviation in the ischium scan (ISCSTD and ISCSTSD), soft tissue density in the 8th thoracic vertebra scan and its standard deviation (TV8STD and TV8STSD). This model (N^{com}, R^2 = 0.67) was then used as a maximum benchmark and the thirteen remaining models also included in the validation analysis were tested against the maximum benchmark using Fisher's z transformation (Rasch et al., 1978). All of the models performed as well as the maximum benchmark model in the validation analysis ($P < 0.05$; R^2 = 0.59 to 0.66). This left fourteen models for consideration as predictors of IMF, five of which used SCTS information and nine which used a combination of SCTS information and reference information. Details of the final selected prediction models developed from the entire data set are presented in Table 6. These included Models B^{sp}, F^{sp}, H^{sp}, J^{sp} and L^{sp} using SCTS information and models B^{com}, F^{com}, H^{com}, J^{com}, K^{com}, L^{com}, N^{com} , O^{com} and P^{com} using a combination of information from both the reference scans and SCTS.

Table 6: Final prediction models and equations derived from the whole data set, adjusted coefficient of determination (Adj R^2) and residual mean square error of the prediction (RMSEP)

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

^{sp} Model uses information from spiral scans only

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

4. Discussion

It has been demonstrated in previous studies that information from single or multiple CT scans can provide moderately accurate predictions of IMF in different sheep breeds. Prediction accuracies range from R^2 = 0.33 to 0.68. (Clelland et al., 2014; Karamichou, Richardson, Nute, McLean, & Bishop, 2006; Lambe, McLean, et al., 2010; J. Macfarlane, Lewis, Emmans, Young, & Simm, 2006) These studies have provided evidence of the potential use of single-slice CT scanning as a predictor of IMF in different sheep breeds. The results from this study provide evidence that further improvements in the prediction of IMF are possible and the use of information from both spiral CT scans and a combination of spiral CT scans and reference scans can adequately predict intramuscular fat content in the loin of purebred Texel sheep.

Prediction models using CT parameters in the assessment of IMF content, achieved a maximum accuracy of AdjR^2 = 0.70 and 0.71, using either spiral information only, or a combination of spiral and reference scan information respectively. The results from this study indicate that there are several potential prediction models that may be developed, using different combinations of CT parameters. There was a group of potential prediction models with increasing degrees of complexity that had similar prediction accuracies for IMF, which could be indicative of a possible 'ceiling' in the achievable prediction accuracies we may expect using these types of CT parameters. Models that included increasing numbers of independent variables appeared to be slightly less transferable when validated against the independent time series data. Although not significant, the models including fewer independent variables and more direct measures of soft tissue density (average and standard deviation) were generally more robust during validation. This suggests that the complexity of the model may have an effect on the accuracy of prediction when applied to an independent data set.

Given that there are few, if indeed any, *in vivo* predictors of MQ traits in meat producing species, prediction accuracies may be acceptable with a suggested lower limit of R^2 = 0.30.

However in this study the use of CT parameters failed to adequately estimate shear force of the loin producing an upper limit of R^2 = 0.13. Similar studies carried out by Lambe et al. (2008) and Karamichou et al. (2006) reported low phenotypic correlations between two dimensional CT parameters and shear force (r = 0.15 – 0.22, r = 0.16 respectively). Although IMF is regarded as an important factor in the eating quality of meat when related to mouth feel, tenderness, juiciness and species-specific flavor, the relationship between shear force and IMF is less clear. Other factors such as cooking loss, ultimate pH, post-mortem glycolosis and conditioning (ageing) play an important role in the conversion of muscle to meat and may have significant effects on shear force results. The CT parameters of the same muscle *in vivo* to that of a processed, aged and cooked piece of meat may be too far removed for shear force parameter estimation or prediction to be possible. There is evidence of a linear relationship between shear force values in cooked meat samples and solvent-extracted IMF content in raw meat samples and it is generally accepted that this relationship exists (Hopkins, Hegarty, Walker, & Pethick, 2006; Pannier et al., 2014; Safari, Fogarty, Ferrier, Hopkins, & Gilmour, 2001), although the size of the effect is often debated.

Breeding programs in the UK for several species of livestock have resulted in substantial genetic improvement in areas such as production efficiency. Genetic improvement in such traits are permanent and cumulative (Simm, 1998). CT predictions of carcass fat and muscle weights and muscularity in both the gigot and loin have been used in pedigree UK sheep breeding programmes over the last two decades (L. Bünger, J.M. Macfarlane, N. R. Lambe, J. Conington, K. A. McLean, K. Moore, 2011). Together with ultrasound measures of fat and muscle depth in the loin region, CT measured carcass fat and muscle weights have contributed much to the success of breeding for leaner carcasses (Moore, McLean, & Bunger, 2011). However, it remains that the drive for reduced carcass fatness and increased muscularity in current breeding programmes is having an impact on IMF content and as a result meat eating quality traits (Pannier et al., 2014). This study shows that there may be several approaches using SCTS technology to predict IMF as a MQ trait and a proxy for meat eating quality traits.

In conclusion, the prediction of mechanical shear force could not be achieved at an acceptable level of accuracy employing information from SCTS information, or a combination of reference scan image information and SCTS information. However, the prediction of IMF in the loin employing information from SCTS with or without additional information from reference scans was more promising. This study provides valuable evidence that the prediction of IMF and related meat eating quality traits for Texel lambs *in vivo* can be achieved using spiral x-ray CT technology. However the increase in accuracy when employing SCTS technology was not significant when compared to previous studies using single slice scanning procedures ($P < 0.05$; Clelland et al., 2014). This suggests that the use of SCTS technology in the prediction of IMF does not adequately increase prediction accuracies to justify additional image analysis involved in the processing of the resulting data. Therefore the authors conclude that although the methods used in this study were successful in the prediction of IMF, the increased image analysis and processing currently required does not justify the increase in accuracy achieved when compared to current reference scan procedures.

Acknowledgements

Funding for this work was gratefully received from AHDB Beef and Lamb, Quality meat Scotland and HCC as part of Neil Clelland’s PhD studies. Thanks go to Ian Richardson and colleagues at the University of Bristol for performing laboratory tests as part of the historical trials.

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Figure 1: Detailed tomogram's, single slice and spiral images produced during CT scanning
(a) First image where TPLV7 appears (i), last image where TPLV1 is no longer visible (ii) and 3D rendered stack of selected images (iii)
(b) Scan image from ischium region (i) and scan image from 8th thoracic vertebra region (ii)

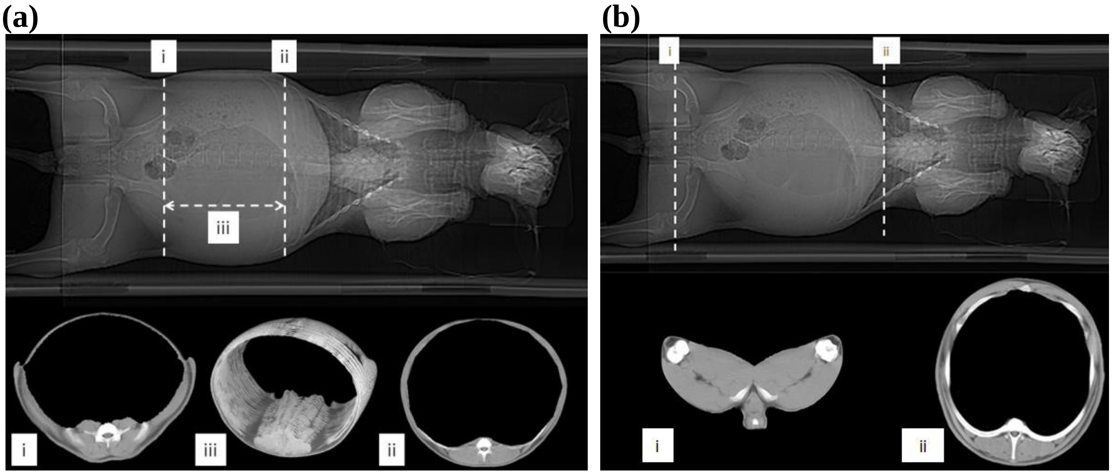

Figure 2: Histograms of chemically extracted intramuscular fat percentage (IMF %) and shear force (kgF) measured in the loin of the Texel lambs (n = 370)

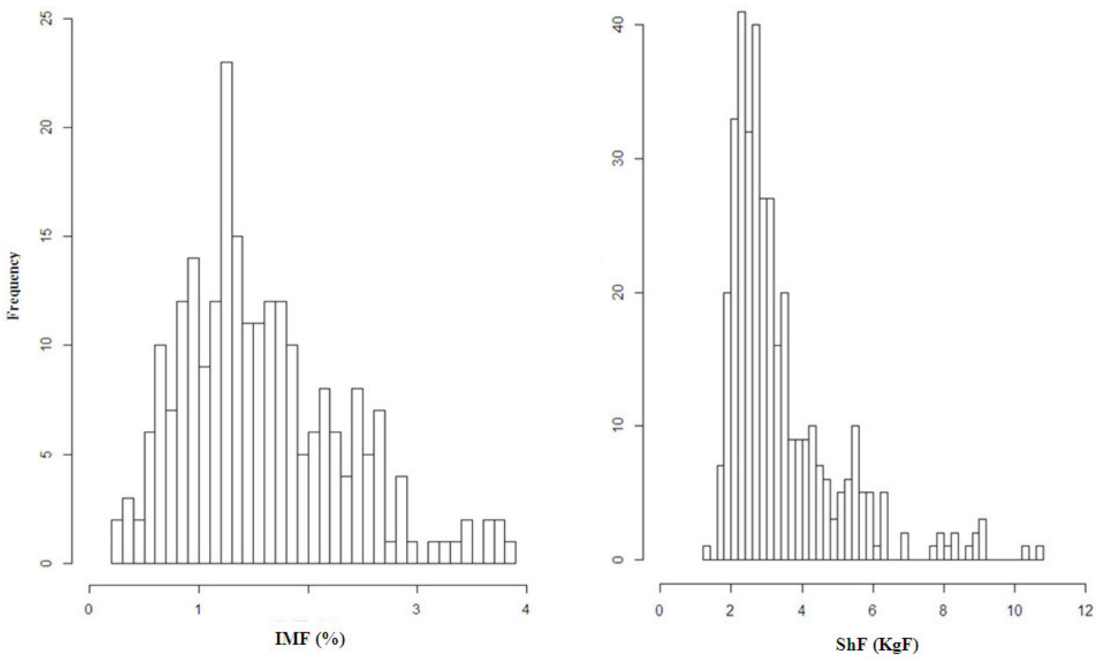

Figure 3: Histogram of chemically extracted intramuscular fat percentage (IMF %) and shear force (kgF) both measured in the loin in the calibration and validation data sets