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Prediction of intramuscular fat content using CT scanning of packaged lamb cuts and relationships with meat eating quality

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Abstract

Novel, multi-object X-ray computed tomography (CT) methodologies can individually analyse vacuum-packed meat samples scanned in batches of three or more, saving money and time compared to scanning live animals. If intramuscular fat (IMF), as a proxy for meat quality, can be predicted with similar accuracies as in live lambs, this method could be used to grade on quality, or to inform breeding programmes. Lamb loin cuts from commercial carcasses (n=303), varying in fat and conformation grade, were vacuum-packed and CT scanned, then tested for meat quality traits and by a trained taste panel. Tissue density values measured by CT, alongside carcass and loin weights, predicted IMF with moderate accuracy (R^2 0.36), but did not accurately predict shear force or sensory traits. Juiciness and flavour increased linearly with IMF, whilst texture and overall liking increased to an optimum between 4 and 5% IMF. Samples predicted by CT as having >3% IMF scored significantly higher for sensory traits, than those predicted as <3% IMF.

Keywords

Lamb; meat quality; intramuscular fat; computed tomography

1. Introduction

Experimental work over the last two decades (Bunger, Moore, McLean, Kongsro & Lambe, 2014) has provided underpinning scientific evidence for the best way to incorporate x-ray computed tomography (CT) data for carcass traits into UK meat sheep breeding programmes. This work has included development of scanning protocols and image analysis software (Mann, Young, Glasbey, McLean, Navajas & Bunger, 2013), calibration with dissection (Lambe, Young, McLean, Conington & Simm, 2003; Macfarlane, Lewis, Emmans, Young & Simm, 2006), and development of estimated breeding values (EBVs) for CT traits (e.g. Jones, Lewis, Young & Simm, 2004). Computed tomography provides highly accurate (>90%) estimates of carcass tissue weights (Young, Lewis, McLean, Robson, Fraser, Fitzsimons et al., 1999; Young, Simm & Glasbey, 2001) and increases rates of genetic gain (by 7% for muscle weight, 10% for fat weight and 20% for muscularity; Moore, McLean & Bunger, 2011) over those achieved using ultrasound alone. Computed tomography scanning has been used in UK terminal sire breeding programmes since 2000 to accurately estimate carcass composition and muscularity (Bunger et al., 2014). However, the wealth of information provided by CT scanning means that many other carcass traits can be accurately predicted or measured using the images and data produced. For example, partitioning and distribution of fat and muscle depots can be assessed.

Intramuscular fat (IMF) is important due to its association with meat eating quality (sensory) traits, such as juiciness and flavour. A minimum level of 3% IMF in grilled cuts of red meat such as beef and lamb was recommended by Savell and Cross in 1988 to ensure consumer acceptability in terms of eating quality. However, a more recent study in Australia recommended a minimum of 5% IMF (Hopkins, Hegarty, Walker & Pethick, 2006) in lamb meat. The ability of CT to predict IMF has been confirmed in a number of studies in live lambs of different breeds (Young et al., 2001; Karamichou, Richardson, Nute, McLean & Bishop, 2006; Macfarlane, Lewis, Emmans, Young & Simm, 2009; Navajas, Lambe, Bünger, Glasbey, Fisher, Wood et al., 2006; Lambe, Navajas, Schofield, Fisher, Simm, Roehe et al., 2008; Clelland, Bunger, McLean, Conington, Maltin, Knott et al., 2015) and in live pigs (Lambe, Wood, McLean, Walling, Whitney, Jagger et al., 2013), providing a rare *in-vivo* predictor of meat quality. Most of these studies found the strongest CT predictors of IMF to be average density values of the pixels in the CT images associated with muscle and, in some cases, fat. Recently, the first genetic parameters for CT-predicted IMF were estimated and moderate heritabilities reported (Clelland, Bunger, McLean, Knott & Lambe, 2015). Muscle density measured by CT has also been shown to be moderately correlated (genetically and phenotypically) with sensory eating quality traits (Karamichou et al., 2006; Lambe, Navajas, Fisher, Simm, Roehe & Bunger, 2009). Therefore, CT has the capability to simultaneously select sheep for higher eating quality (IMF) and lower waste (carcass trim-able fat).

Computed tomography is non-invasive and non-destructive, so can be used on cuts of meat for human consumption, which would reduce the costs and impracticalities associated with scanning live animals. This could make selection for meat quality feasible, if suitable predictors can be assessed in meat cuts and fed back to breeding programmes, or could be used to sort lamb cuts into quality grades based on meat quality characteristics.

Although the link between CT predictors and IMF is well-established in live lambs, evidence is more limited on the ability to predict IMF from CT of lamb meat cuts. Results from previous studies on cuts of pork (Furnols, Brun, Tous & Gispert, 2013) and beef (Prieto, Navajas, Richardson, Ross, Hyslop, Simm et al., 2010) suggest that it could be successful, even using CT of vacuum-packaged meat (Prieto et al., 2010), although distribution of IMF differs in lamb compared to beef or pork. Methodologies to allow multi-object CT scanning (up to 6 samples scanned simultaneously) have recently been developed by SRUC and Biomathematics and Statistics Scotland (BioSS), including routines to allow data handling, storage and image analysis of individually identified samples (Mann et al., 2013; Clelland, Price, Bunger, McLean, Knott, Haresign et al., 2013). Multi-object scanning has the potential to provide accurate results for individually-labelled meat samples, allowing further savings in cost and time compared to scanning animals or meat samples individually.

This study aimed to test the ability of multi-object CT scanning to predict IMF and other meat (eating) quality traits in vacuum-packed cuts of lamb meat from the loin. The relationships between IMF and sensory meat eating quality traits were also investigated, to assess the ability of taste panels to differentiate between IMF levels and potentially identify a UK-relevant window of IMF acceptability for lamb.

2. Material and methods

2.1. Loin cut sourcing

On each of three consecutive days in October 2014 (days 1, 2 and 3), approximately 100 lamb carcasses were selected from the commercial slaughter line at Wm Morrison's Woodhead Brothers abattoir in Turriff, Aberdeenshire. A total of 303 loins were collected. The aim was to sample from all fat and conformation classes within the EUROP carcass classification grid, as employed in the UK for carcass grading. Immediately post-slaughter, after dressing, hot carcasses were subjectively classified by a trained grader into conformation classes E, U, R, O, P (E being excellent conformation and P poor conformation) and fat classes 1 (very lean) to 5 (very fat), with fat classes 3 and 4 subdivided into low (L) and high (H). Fat class is expected to correspond to the estimated subcutaneous fat percentage of the carcass: 1 = 4, 2 = 8, 3L = 11, 3H = 13, 4L = 15, 4H = 17 and 5 = 20 (Kempster, Cook & Grantley-Smith, 1986). The distribution of carcasses in the dataset across these classes is shown in Table 1. No carcasses with conformation class P (poor) were available, but the distribution is believed to reflect the typical spread of carcasses through this abattoir during this prime slaughter period.

Table 1: Distribution of loin samples across carcass classes

Fat class/ Conformation class	1	2	3H	3L	4H	4L	5	Total
E		2	17	18	1	12		50
U	1	15	44	34	17	50	2	163
R		22	16	24	7	16		85
O	1			4				5
P								
Total	2	39	77	80	25	78	2	**303**

From the saddle, the flank was removed and the lumbar region of the loin (M. *Longissimus lumborum* (LL)) including both sides of the carcass, bone-in, was collected and vacuum-packed using a SEALED AIR rotary vacuum packer that pulls a 3mb vacuum. SEALED AIR Cryovac, high barrier, multilayer bags were used, specifically designed for bone-in packaging . The temperature of product as packing was 4-6^{o}C. Each animal was individually traced to align slaughter performance with the traits measured on the loin. The vacuum-packed loin cuts were chilled and delivered by refrigerated transport (1-3^{o}C) to the CT scanning unit at SRUC, Edinburgh, on day 5. Maintaining chilled conditions, the vacuum-packed loin cuts were weighed, then CT scanned on days 8, 9 and 10, so that all samples were scanned between 7 and 8 days *post-mortem*.

2.2 Computed tomography scanning

The loin cuts were scanned using CT protocols specifically designed for scanning cuts of meat, which have been described in detail in previous studies (Clelland et al., 2013). Loins were uniformly orientated and positioned on a multiplex scanning frame and spiral CT scanned (contiguous scans at 8mm intervals) in batches of three.

The amount of absorption of x-rays during CT scanning depends on tissue density and can be quantified using CT numbers (relating to greyscale values in the resulting image) or Houndsfield units (HU). The CT scanner is calibrated to assign water a value of 0 HU and air a value of –1000 and different tissues can be assigned values ranging from –1000 to +1000 HU (Wegner, 1993). Density values of each pixel can be denoted either as greyscale values or as Hounsfield units, after undergoing a linear transformation. Computed tomography images were segmented using a multi-object animal tomograph analysis routine (ATAR) software, developed at BioSS/SRUC (Mann et al., 2013). From the density value assigned to each pixel in each image by the CT scanner, pixels were allocated as fat, muscle, or bone, using previously-developed density thresholds, specific to the analysis of images obtained from carcasses, primal cuts and dissected muscles (Table 2). Tissue weights were calculated using area and density (converted to g/cm^3) values for each tissue. The CT density results for each tissue were then weighted by area in each image and averaged over the spiral series images (26-30 images per loin, average = 28 images) to give an overall average density value (and its standard deviation) for fat and muscle. Additionally, the pixels allocated as fat and muscle were combined to give overall "soft tissue" weights and density values (Table 2).

Table 2: Tissue density thresholds (expressed in both greyscale and Houndsfield units) applied to the CT images to allocate all pixels to fat, muscle, bone, or soft tissue

Threshold	Greyscale values	Hounsfield units (HU)
Minimum fat /soft tissue	6	-244
Maximum fat	140	24
Minimum muscle	141	26
Maximum muscle / soft tissue	230	204
Minimum bone	231	206

For each loin sample, a histogram, or frequency distribution, was also constructed from results obtained from the ATAR software, quantifying the number of pixels allocated to each greyscale (density) value (0-254), across all images in the spiral series.

2.3 Intramuscular fat extraction

Following CT scanning, the loin cuts were unpackaged and cut into different sections for further testing. From the cranial end, 3cm of the left side LL was removed for fatty acid analysis, the next 6cm of this muscle on the left side was removed for mechanical tenderness assessment, whilst the full right side LL was allocated to taste panel analysis. All sections were frozen and sent to the University of Bristol for laboratory testing.

Samples were later thawed and fatty acid analysis was carried out by direct saponification as described in detail by Teye et al. (Teye, Sheard, Whittington, Nute, Stewart & Wood, 2006). Samples were hydrolysed with 2M KOH in water:methanol (1:1) and the fatty acids extracted into petroleum spirit, methylated using diazomethane and analysed by gas liquid chromatography. Samples were injected in the split mode, 70:1, on a CP Sil 88, 50m30.25mm fatty acid methyl esters (FAME) column (Chrompack UK Ltd, London, UK) with helium as the carrier gas. The output from the flame ionization detector was quantified using a computing integrator (Spectra Physics 4270; Darmstadt, Germany) and linearity of the system was tested using saturated (FAME4) and monounsaturated (FAME5) methyl ester quantitative standards (Thames Restek UK Ltd, Windsor, UK). All measurements of fatty acids were performed in duplicate, the error between replications being usually 1% to 2% with a maximum allowance of 5% error. Individual fatty acids were not considered for this paper, but will be the subject of further analysis. Total IMF content was calculated gravimetrically as total weight of fatty acids extracted, which was the trait of interest for the current study.

2.4 Mechanical tenderness

The thawed loin sample was cooked 'sous vide' in a polythene bag submerged in a temperature controlled water bath (80^{o}C). Core temperature of the loin was constantly monitored until an internal core temperature of 75^{o}C was reached. Loins were rapidly cooled and stored under refrigeration for up to 24 hours. Loin samples were then prepared for shear force testing by cutting ten 10mm x 10mm x 20mm core samples from each loin. Samples were sheared using a MIRINZ tenderometer (bite test) to assess the force required to shear through the sample (Bickerstaffe, Bekhit, Robertson, Roberts & Geesink, 2001). Average peak force (ShF) was recorded as the mean peak force across a maximum of ten samples for each loin.

2.5 Taste panel evaluation

Sensory evaluations of meat eating quality (sensory) were performed by a trained taste panel at the University of Bristol, according to previously-described protocols (Nute, 2002). For the sensory evaluation, samples were thawed at 4^{o}C overnight. They were then cut into 2 cm thick steaks and cooked in a contact grill (George Foreman Double Knockout grill, model 10635) until the internal temperature reached 75^{o}C, measured by a thermocouple inserted into the geometric centre of the sample. Between 6 and 10 assessors rated 2cm x 2cm x 2cm samples of each muscle. The assessors used 8-point category scales (Sanudo, Nute, Campo, Maria, Baker, Sierra et al., 1998), to evaluate the following traits:

texture (1 = extremely tough, 8 = extremely tender); juiciness (1 = extremely dry, 8 = extremely juicy); lamb flavour intensity (1 = extremely weak, 8 = extremely strong), abnormal flavour intensity (1 = extremely weak, 8 = extremely strong) and overall liking (1 = dislike very much, 8 = like very much). Therefore, higher scores represented better sensory in all traits except for abnormal flavour, where lower scores were preferred by the assessors.

2.6 Statistical analysis

Multiple ordinary linear regression (OLR) analyses were performed to predict objective (IMF and ShF) and sensory (texture, flavour, juiciness and overall liking) meat quality traits, using CT summary parameters, as well as weights of the cold carcass and loin cut (Table 3). From this list of possible predictor traits, the final model was selected using the stepwise GLM procedure in Genstat (Payne, Murray, Harding, Baird & Soutar, 2013), which includes or excludes terms from a multiple ordinary linear regression model according to the ratio of residual mean squares.

Table 3: Parameters tested in the GLM models to predict meat quality and meat eating quality traits using CT derived traits

Acronym	Description	Acronym	Description
CCWT	Cold carcass weight measured morning after slaughter (kg)	FD	Average density of fat pixels in all cross-sectional scans weighted by fat area (HU)
LoinWT	Weight of loin cut measured after 7 days aging (g)	MD	Average density of muscle pixels in all cross-sectional scans weighted by fat area (HU)
FWT	Weight of fat estimated by CT (g)	STD	Average density of soft tissue pixels in all cross-sectional scans

			weighted by fat area (HU)
MWT	Weight of muscle estimated by CT (g)	FSD	Standard deviation of fat density in all cross-sectional scans weighted by fat area (HU)
STWT	Weight of soft tissue (fat + muscle) estimated by CT (g)	MSD	Standard deviation of muscle density in all cross-sectional scans weighted by fat area (HU)
CTLoinWT	FWT + MWT + bone weight as estimated by CT (g)	STSD	Standard deviation of soft tissue density in all cross-sectional scans weighted by fat area (HU)
%FatCT	FWT/CTLoinWT		

After performing OLR analyses with the full data set, to determine the optimal model terms, the data were then divided into a calibration data set (including the samples from the first two slaughter days) and a validation data set (including the samples from the third slaughter day) to test the predictive ability of the model. Prediction equations were derived from the calibration data and then applied to the validation data. True validation would require testing of prediction equations in a completely independent dataset, however, no such dataset was available. Therefore, available data were split using a natural time series separation (Snee, 1977), to provide some indication of the transportability of these prediction equations.

For meat quality traits where promising accuracies of prediction were obtained, the ability of CT to sort lamb cuts into potential meat quality grades was assessed. Predicted meat quality was calculated for each sample, using the prediction equations derived from CT parameters, and used to group samples into "bands" of meat quality, which were compared to similar groupings defined by laboratory-estimated meat quality values. These bands maybe more relevant if the aim was to use this technology for grading or sorting of meat cuts for different markets, based on meat quality.

A second regression method, partial least squares regression (PLSR), was then used in Genstat (Payne *et al.*, 2013) to investigate whether more accurate predictions of meat quality could be made by considering the frequency distributions of pixel density values (on the greyscale). Numbers of pixels of each greyscale value (summed across all 2-dimensional CT images in the spiral scan series) were used as predictor variables (X) and IMF and other meat quality traits as predicted variables (Y). PLSR is particularly suited when the matrix of predictors has more variables than observations, and when there is multicollinearity among X values. PLSR considers linear combinations of the independent variables as factors and adds successive factors to both minimise the residuals and simultaneously to have high squared covariance with Y variables. Multiple factors (or dimensions) are constrained to be mutually orthogonal. For this dataset, cross-validation was performed (using 3 groups, split by slaughter day) in order to choose the correct number of dimensions of histogram values to explain each meat quality trait without over-fitting the PLSR equations. The optimal number of dimensions in each equation was determined in Genstat using the predictive residual error sum of squares (PRESS) and Osten's F-test, which tests the significance of incremental changes in PRESS (Osten, 1988).

To determine the relationships between IMF (or CT-predicted IMF) and sensory traits, regression analyses (linear and polynomial to order 3) were performed in Genstat. To assess the potential for consumers to differentiate between meat samples sorted into different IMF grades or bands (either by chemical analysis or CT prediction equation), IMF values were also grouped into bands and fitted as a factor in a regression model in Genstat, to explain variation in sensory traits.

3. Results

Figures 1a and 1b show the frequency distribution for chemically extracted IMF and ShF. Values of meat quality traits >3 standard deviations from the mean were considered outliers and were removed from the data set. This included 3 values for IMF, 4 for ShF and 2 each for texture, flavour and overall liking. Three records were also removed that had outlying values for each of the CT density traits FD, MD, FSD, MSD. Table 4 summarises the remaining data set, used for analysis, considering the full data set, the calibration data set (slaughter days 1 and 2) and the validation data set (day 3).

Insert Figures 1a and 1b here

Table 4: Summary statistics for meat (eating) quality traits in the full data set, the calibration data set and the validation data set

	All data (n=300)[1]				Calibration (n=200)[1]		Validation (n=100)[1]	
	Mean	s.d.	min	max	Mean	s.d.	Mean	s.d.
Meat quality traits								
IMF (%)	3.12	0.85	1.72	6.97	2.98	0.78	3.09	0.77
ShF (N)	29.5	7.6	13.9	53.7	31.6	8.9	28.6	6.5
Texture	5.71	0.55	4.00	6.89	5.68	0.57	5.78	0.49
Flavour	5.38	0.38	4.22	6.33	5.36	0.39	5.41	0.38
Juiciness	5.07	0.38	4.22	6.11	5.07	0.38	5.06	0.39
Liking	5.19	0.41	4.00	6.22	5.17	0.41	5.21	0.40
Predictor traits[2]								
CCWT	20.8	2.1	14.7	26.0	20.4	2.0	21.5	2.1
LoinWT	1.53	0.21	1.00	2.04	1.51	0.20	1.57	0.22
FWT	0.43	0.13	0.16	0.75	0.42	0.12	0.46	0.14
MWT	0.79	0.11	0.44	1.19	0.78	0.11	0.79	0.11
STWT	1.22	0.18	0.75	1.67	1.20	0.18	1.25	0.20
%FatCT	30.1	6.5	14.6	47.6	29.6	6.1	31.0	7.3
FD	-69.3	6.9	-84.2	-48.4	-69.6	6.8	-68.7	7.0
MD	68.6	3.3	61.4	75.7	68.4	3.1	69.0	3.5
STD	15.4	12.8	-15.9	46.9	15.9	12.1	14.3	14.1
FSD	57.0	5.9	45.0	73.7	57.6	5.4	55.8	6.5
MSD	25.3	1.0	22.6	29.5	25.3	1.0	25.4	1.1
STSD	75.2	5.4	60.3	85.3	75.3	5.2	75.1	5.7

[1]Number of IMF records, the number of records for other traits may vary by a maximum of 4
[2]See Table 3 for trait descriptions and units

3.1 Prediction of meat quality traits using CT

Correlations were calculated between each meat quality trait and each predictor trait (Table 5) as a first indication of the most valuable predictors to include in multiple regression models. Results showed that the single predictor trait most highly correlated with each of the meat quality traits was %FatCT (the proportion of the pixels in the loin cut images allocated as fat, which included pixels within the intramuscular, intramuscular and subcutaneous fat depots).

Table 5: Correlations (r) between meat (eating) quality traits and model parameters. Values in bold are significantly different from zero.

	IMF	ShF	Texture	Flavour	Juiciness	Liking
CCWT	**0.23**	0.00	0.01	0.03	0.06	0.03
LoinWT	**0.26**	0.02	0.03	0.02	0.08	0.03
FWT	**0.53**	-0.09	**0.21**	**0.17**	**0.17**	**0.18**
MWT	**-0.16**	0.08	**-0.13**	**-0.15**	-0.08	**-0.16**
STWT	**0.27**	-0.01	0.06	0.03	0.08	0.03
%FatCT	**0.59**	**-0.13**	**0.26**	**0.23**	**0.21**	**0.25**
FD	**-0.35**	0.09	**-0.20**	-0.07	-0.02	**-0.12**
MD	**-0.14**	-0.04	0.01	-0.02	**-0.13**	0.00
STD	**-0.58**	0.11	**-0.26**	**-0.22**	**-0.20**	**-0.24**
FSD	**-0.49**	**0.13**	**-0.21**	**-0.19**	**-0.13**	**-0.20**
MSD	0.10	-0.03	0.09	0.09	0.11	0.07
STSD	**0.50**	**-0.13**	**0.24**	**0.17**	**0.13**	**0.21**

Some parameters in the list of possible predictor traits were very highly correlated with each other (r >0.85): STWT with CTLoinWT and LoinWT; FWT with %FatCT, STD and FSD; STD with %FatCT; STSD with FD and STD. These terms were, therefore, not fitted together in the multi-variate regression analysis to avoid auto-correlation.

The best prediction models derived by stepwise OLR for each of the meat quality traits are shown in Table 6. Prediction accuracies are shown for the full data set (using prediction equations derived from all data), the calibration data set (using prediction equations derived from slaughter days 1 and 2 only) and the validation data set (using prediction equations derived from slaughter days 1 and 2). Of the meat quality traits tested, the CT parameters predicted IMF with the highest accuracy, accounting for 36.3% of the variation observed across all loin samples. Around 10% of the variation in overall liking was explained by CT parameters, but <10% of variation in the other meat quality traits. Splitting the data in to calibration and validation data sets resulted in similar prediction accuracies as those obtained using the full data set, across traits (Table 6).

Table 6: Prediction accuracy of meat (eating) quality traits using the best models in OLR, considering the full data set, calibration data set (slaughter days 1&2) and validation data set (slaughter day 3)

	All data	Calibration	Validation	
Trait	Adj-R^2	Adj-R^2	R^2 (RMSEP)	Terms in model

	(RMSE)	(RMSE)		
IMF	0.363 (0.62)	0.337 (-0.64)	0.393 (-0.6)	CCWT + LoinWT + FD + MD + STD
ShF	0.025 (-0.83)	0.018 (-0.9)	0.004 (-0.66)	%FatCT + LoinWT + MWT + MD
Texture	0.082 (-0.53)	0.081 (-0.55)	0.062 (-0.47)	%FatCT + LoinWT + MD
Flavour	0.091 (-0.37)	0.07 (-0.38)	0.109 (-0.36)	LoinWT + FD + MD + STD + MSD
Juiciness	0.059 (-0.37)	0.056 (-0.37)	0.054 (-0.38)	FD + STD
Liking	0.098 (-0.39)	0.086 (-0.39)	0.092 (-0.38)	MWT + STWT + FD + MD + STD + FSD + MSD

The best prediction equation for IMF%, using the full data set, was:

IMF% = 2.897 + (0.0797*CCWT) - (0.000692*LoinWT) + (0.02477*FD) + (0.029*MD) - (0.0488*STD)

Model 1

Using this prediction equation, the ability of CT to categorise lamb cuts into different IMF bands was investigated, to assess its potential for sorting into different grades of meat quality. The accuracy of prediction for the other meat quality traits was considered too low to make similar analyses worthwhile for those traits. Categorising IMF into 5 percentage bands, the actual chemical IMF band was matched to the predicted IMF band (when IMF was predicted using Model 1). The frequency of samples assigned to each band is shown in Table 7. In total, 53.5% of samples were correctly assigned to band (shown in bold, Table 7). The majority (63.3%) of samples with chemical IMF < 3% were assigned a band below 3-4% by CT, however, 25.2% of those with IMF > 3% were assigned as below band 3-4%. Of the samples with IMF > 4% (n=29), only 1 sample was assigned as greater than band 3-4%. These results suggest that the CT prediction equation is less good at identifying samples in the bands at the high end of the IMF distribution.

Table 7: Frequency of samples in each IMF percentage band[1]

	CT-predicted IMF converted to % band				
IMF % band	**1-2%**	**2-3%**	**3-4%**	**4-5%**	**Total**
1-2%	**5**	17			22
2-3%	3	**70**	55		128
3-4%		34	**83**	1	118
4-5%		2	23	**1**	26

>5%		1	2		3
Total	8	124	163	2	297

[1]Where IMF value was an integer, this was included in the higher band (e.g. IMF = 2% was included in band 2-3%)

Using the second regression method of PLSR, considering the frequency distribution of pixel densities across all CT images within a sample, results (Table 8) suggest that there were no substantial improvements over OLR (Table 6) in prediction accuracy. For IMF in particular, prediction accuracy was reduced by ~11%.

Table 8: Prediction accuracy of meat (eating) quality traits using the best models in PLSR

Trait	R^2	RMSECV	No. of dimensions in model
IMF	0.253	0.753	7
ShF	0.041	0.842	3
Texture	0.125	0.542	6
Flavour	0.050	0.379	1
Juiciness	0.050	0.376	1
Liking	0.075	0.398	1

3.2 Relationships between IMF and sensory traits

Linear and polynomial regressions were tested between IMF and sensory traits (including outliers). Fitting a quadratic regression accounted for the largest proportion of variation in texture (R^2 = 8.6%) and overall liking (R^2 =11%) (Figure 2), suggesting that these traits peaked between 4 and 5% IMF. However, a linear regression gave the best fit between IMF and the sensory traits of flavour (R^2 = 10%) and juiciness (R^2 = 5.7%) (Figure 2), implying that these traits continued to rise with IMF across the range of IMF values observed.

Insert Figure 2 here

Regressing CT-predicted IMF on sensory scores, gave slightly lower accuracies of prediction (7% for texture and flavour, 5% for juiciness and 8% for overall liking) and, for these relationships, fitting non-linear trend lines did not improve fit over the linear relationships.

3.2.1. Chemical IMF results. To assess the ability of consumers to differentiate between meat samples assigned into "bands" of meat quality, IMF values were grouped into bands, as above, and the relationship with variation in sensory traits tested (Figure 3). Significant differences were observed in sensory traits between IMF bands. Samples in the IMF range 4-5% had the highest mean score for all taste panel traits, except juiciness, where samples with >5% IMF had the highest mean score. Samples with IMF of 4-5% scored significantly higher (P<0.05) than samples with <3% IMF for all traits except juiciness. For all traits except texture, samples with 3-4% IMF scored significantly higher than samples with <3% IMF. Due to the small number of samples with >5% IMF, this category was not significantly different from any other IMF band for any of the sensory traits. Samples in the 3-4% IMF band were not significantly different to those in the 4-5% band for any sensory trait.

Insert Figure 3 here

3.2.2. CT-predicted IMF results To assess the ability of consumers to differentiate between meat samples assigned by CT into "bands" of meat quality, CT-predicted IMF values (using Model 1) were grouped into bands. Values fell into four bands, but there were only 8 records in the 1-2% band and only 2 records in the 4-5% band, so these were incorporated into the other bands, which were then defined as <3% or ≥3% CT-predicted IMF. The results of the GLM model fitting these bands to explain variation in sensory traits are shown in Table 9.

Table 9: Predicted means for meat (eating) quality traits at each CT-predicted IMF band level[1]

		CT-predicted IMF band		
	Adj-R^2	<3%	≥3%	P value
N		132	165	
Texture	0.070	5.55	5.85	<0.001
Flavour	0.038	5.29	5.45	<0.001
Juiciness	0.044	4.98	5.15	<0.001
Liking	0.057	5.08	5.28	<0.001

Samples predicted as having ≥3% IMF, by CT prediction equations, were scored significantly higher on average by the taste panel for each trait, than those predicted as having <3% IMF. The percentage of variance accounted for by these CT-predicted IMF bands was low (7% or less) for each sensory trait using each model. However, these prediction accuracies were similar in magnitude to those obtained using chemical IMF bands in the model.

4. Discussion

A number of studies on different breeds have confirmed that CT measurements of muscle and fat densities can predict IMF in live lambs with moderate to high accuracy (Young et al., 2001; Karamichou et al., 2006; Macfarlane et al., 2009; Lambe et al., 2008; Clelland et al., 2015). Published prediction accuracies in live lambs differ between studies and tend to fall in the range of 33-70% (Clelland, 2015), probably due to differences in structure of the study population and methods used. Less information is available on relationships between meat quality traits and CT measurements taken from primal cuts. Tissue densities change after slaughter (due to blood loss, chilling etc.), therefore established relationships and protocols developed for the live animal may not be transferable to carcass joints. A study on a small number (30) of dissected lamb LL muscles (Lambe, McLean, Macfarlane, Johnson, Jopson, Haresign et al., 2010) suggested that promising correlations between IMF and CT tissue density values also exist post-mortem, as IMF was predicted with an accuracy of 44% using similar CT parameters as those tested in the OLR analyses in the present study. The prediction accuracy found by OLR in the current study, using vacuum-packed loin cuts, is of a similar magnitude as these literature estimates for lamb. A lower accuracy (20%) was reported by Clelland et al. (2013) when predicting IMF in dissected strip loins with CT parameters, although this may have been caused by changes in muscle structure due to freezing and thawing of the samples before CT scanning, disturbing relationships between IMF and tissue density parameters.

Previous studies in beef and pork have used a partial least squares regression (PLSR) approach to estimate IMF from CT data (Prieto et al., 2010; Furnols et al., 2013; Kongsro & Gjerlaug-Enger, 2013). In the work by Furnols et al. (2013), OLR, using 3-6 independent variables describing relative volumes relating to density ranges, was found to predict IMF in pork loins with similar or greater accuracy than PLSR that used many more variables, especially where IMF levels were low. However, both types of models resulted in higher prediction accuracies (R^2 = 0.63-0.83) than have been achieved in the current study on lamb. In a study by Kongsro et al. (2013), PLSR was not considered a feasible method to predict IMF in pig loins *in vivo*, due to low prediction accuracies and high prediction errors, although limited variation in IMF in the sample population is likely to have contributed to these results. The PLSR approach was also previously considered for predicting IMF from CT in live lambs (Lambe, Jopson, Navajas, McLean, Johnson & Bunger, 2009), but was found to be less transportable between data sets/ populations than other prediction equations derived by OLR methods. Furthermore, it is more difficult to assign biological meaning to PLSR results. The prediction equation derived by the OLR method in the current study (Model 1) gave the best prediction of IMF using CT data from lamb primal cuts, but is unlikely to be accurate enough to be used on its own as a means of sorting meat cuts into quality grades or within breeding programmes to select for meat quality.

Analyses of live animal CT images have suggested that poorer predictions of IMF (and unimproved predictions of ShF) are obtained by examining only the muscle tissue in the region where the chemical IMF was measured, and higher accuracies can be achieved by analysing CT images that represent a larger region and incorporate different fat depots, including sub-cutaneous fat (Clelland, 2015). This experiment was therefore designed to use data resulting from CT scanning of entire fresh saddle cuts, with the expectation that this would achieve greater accuracies than using strip loins, as well as allowing more flexibility for further use of the loin cuts as they are returned to the food chain. For this reason, image analysis did not involve selecting smaller regions of interest from within the full CT images obtained. This also suggests that it may be valuable to investigate whether CT scanning of whole carcasses would provide better predictions of meat quality traits than meat cuts, although multi-object CT would not then be possible.

The CT parameters measured in the vacuum-packaged loin cuts were poor predictors of ShF and sensory traits. Low prediction accuracies (R^2 0.03 – 0.14) were previously reported (Clelland, 2015) for mechanical shear force using a range of CT parameters measured in live lambs, across different lamb breeds. Similarly, low correlations ($<\pm0.3$) were observed between CT muscle density, measured *in vivo*, and shear force or taste panel sensory scores, using loin or leg muscles from two divergent lamb breeds, whilst these density measurements had moderate to high negative correlations (-0.3 to -0.7) with IMF (Lambe et al., 2008). These results are also consistent with findings from a study of Scottish Blackface lambs (Karamichou et al., 2006), where low phenotypic correlations (-0.16 to -0.29) were estimated between shear force or taste panel sensory traits and CT muscle density. However, that study was powerful enough to estimate genetic correlations with CT muscle density, which were found to be considerably stronger (-0.49 to -0.8), for all traits except texture assessed by taste panel.

As in other livestock species, IMF in lamb has been shown to have positive effects on consumer sensory scores of tenderness, juiciness, flavour and overall liking (Pannier, Gardner, Pearce, McDonagh, Ball, Jacob et al., 2014). In the current study, IMF accounted for 11% of the variation in overall liking, as assessed by a trained taste panel, and between 5-10% in the other sensory traits. Interestingly, the effects of IMF on texture and overall liking appeared to be curvi-linear, with an optimum IMF level between 4 and 5%, whereas linear positive relationships were observed between IMF and juiciness or flavour, in the lamb samples studied. However, there were low numbers of samples with high IMF levels within the sample population, and few samples with values higher than 5% IMF, so this could not be fully tested and should be interpreted with caution. It would be of interest to confirm these findings using samples representing a greater range of IMF values. Only 3% of the variation in overall liking was explained by IMF in the Australian study by Hopkins et al. (2006), using an untrained consumer panel, where the samples spanned a much wider range of IMF values (2-18%), with a higher mean value of 5.4%. A review of literature to the late 1980s by Savell and Cross (1988) recommended a minimum of 3% IMF for grilled cuts of lamb, such as the loin, to ensure consumer acceptability. A decade later, a study of German Longwool Merino lambs (Heylen, Suess, Freudenreich & von Lengerken, 1998) found that scores of sensory properties increased with IMF in the loin, with IMF content greater than 2.3% resulting in profound improvements in sensory traits, especially flavour and overall acceptance. That study found that sensory characteristics in the LL scored most highly in meat with between 3.5 and 4.5% IMF. However, a minimum of 5% IMF in lamb was recommended based on Australian consumer preferences (Hopkins et al., 2006). These differences may be partially due to experimental design, as well as differences in consumer preferences between countries or across time. The current results, suggesting a peak in overall liking between 4 and 5% IMF, appear to agree with the earlier studies, rather than the Australian study, suggesting geographical differences in preference rather than any consistent changes in consumer preference across recent decades.

Further analysis of the taste panel results (not presented here) suggested that the main sensory trait affecting overall liking was flavour, which accounted for 79% of variation in overall liking when fitted in a simple linear regression, compared to texture, which accounted for 20% and juiciness, which accounted for 14%. No information was known about the breed, age or feeding regime of the lambs that were sampled, all of which are known to affect lamb flavour (Duckett & Kuber, 2001). Greater standardisation of these factors may have made it easier to identify the role of IMF and ShF in overall liking. The specific compounds responsible for lamb flavour have yet to be determined, but fatty acid profiles may play a role (Duckett & Kuber, 2001). The current study collected information on individual fatty acid composition within the samples, the analysis of which was considered outside the remit of this paper and will be the subject of a future study, which could provide some useful insights into factors affecting palatability. Differences in fatty acid composition could also affect fat density, and this could differ depending on storage time and conditions, which could alter some of the CT parameters studied. These factors may be influencing results in the present study and more work is needed in this area to understand these effects.

Given that IMF predicted around 11% or less of the variation in taste panel traits in the lamb samples studied here, and CT parameters directly predicted 10% of the variation in overall liking, the question arises whether we should try to increase accuracies to be able to predict IMF with CT (accuracies currently ~36%) in an effort to improve meat eating quality, or directly predict taste panel (sensory) traits? The sensory traits have lower prediction accuracies, but are the ultimate goal for consumer acceptability. However, scoring of sensory traits is subjective, whereas IMF measurements are objectively measured and should be repeatable. Work in live lambs also suggests that prediction accuracies for IMF using CT could be increased by scanning multiple sections or whole carcasses. The sensory scores in the current study were assessed by a trained taste panel, rather than a consumer taste panel, so may be more consistent, leading to higher prediction accuracies of these traits than have been found in other studies. Selection on predicted values of an objective trait, such as IMF, is likely to be more successful in identifying a consistent product that can be tailored to different markets. Additional samples have been stored from these loins and will be used in future, alongside the lab and taste panel results, to design and conduct a consumer taste panel. This will further assess some of these relationships.

5. Conclusions

Tissue density values, produced by CT scanning vacuum-packed lamb loins, can be used alongside carcass and loin cut weights, to predict IMF with moderate accuracy (using the definitions of Taylor, 1990). However, this method can not accurately predict shear force or sensory traits of the meat. Ordinary linear regression, using tissue density values averaged across all cross-sectional scans, predicts IMF better than partial least squares regression, using frequency distributions of pixels across density values. Sensory traits (texture, flavour, juiciness, overall liking) are significantly influenced by IMF levels: juiciness and flavour increasing linearly with IMF; texture and overall liking increasing with IMF up to an optimum level of between 4 and 5% IMF. Samples predicted as having >3% IMF, by the best CT prediction equation, were scored significantly higher on average by the taste panel for each sensory trait, than those predicted as having <3% IMF. Consumer taste panels should be carried out to further test UK consumer acceptance in lamb meat traits.

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