

Scotland's Rural College

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

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25 **Abstract**

26 Novel, multi-object X-ray computed tomography (CT) methodologies can individually analyse
27 vacuum-packed meat samples scanned in batches of three or more, saving money and time
28 compared to scanning live animals. If intramuscular fat (IMF), as a proxy for meat quality, can
29 be predicted with similar accuracies as in live lambs, this method could be used to grade on
30 quality, or to inform breeding programmes. Lamb loin cuts from commercial carcasses (n=303),
31 varying in fat and conformation grade, were vacuum-packed and CT scanned, then tested for
32 meat quality traits and by a trained taste panel. Tissue density values measured by CT, alongside
33 carcass and loin weights, predicted IMF with moderate accuracy (R^2 0.36), but did not accurately
34 predict shear force or sensory traits. Juiciness and flavour increased linearly with IMF, whilst
35 texture and overall liking increased to an optimum between 4 and 5% IMF. Samples predicted by
36 CT as having >3% IMF scored significantly higher for sensory traits, than those predicted as
37 <3% IMF.

38

39 **Keywords**

40 Lamb; meat quality; intramuscular fat; computed tomography

41

42 **1. Introduction**

43 Experimental work over the last two decades (Bunger, Moore, McLean, Kongsro & Lambe,
44 2014) has provided underpinning scientific evidence for the best way to incorporate x-ray
45 computed tomography (CT) data for carcass traits into UK meat sheep breeding programmes.
46 This work has included development of scanning protocols and image analysis software (Mann,
47 Young, Glasbey, McLean, Navajas & Bunger, 2013), calibration with dissection (Lambe, Young,
48 McLean, Conington & Simm, 2003; Macfarlane, Lewis, Emmans, Young & Simm, 2006), and
49 development of estimated breeding values (EBVs) for CT traits (e.g. Jones, Lewis, Young &
50 Simm, 2004). Computed tomography provides highly accurate (>90%) estimates of carcass
51 tissue weights (Young, Lewis, McLean, Robson, Fraser, Fitzsimons et al., 1999; Young, Simm
52 & Glasbey, 2001) and increases rates of genetic gain (by 7% for muscle weight, 10% for fat
53 weight and 20% for muscularity; Moore, McLean & Bunger, 2011) over those achieved using
54 ultrasound alone. Computed tomography scanning has been used in UK terminal sire breeding
55 programmes since 2000 to accurately estimate carcass composition and muscularity (Bunger et

56 al., 2014). However, the wealth of information provided by CT scanning means that many other
57 carcass traits can be accurately predicted or measured using the images and data produced. For
58 example, partitioning and distribution of fat and muscle depots can be assessed.

59
60 Intramuscular fat (IMF) is important due to its association with meat eating quality (sensory)
61 traits, such as juiciness and flavour. A minimum level of 3% IMF in grilled cuts of red meat such
62 as beef and lamb was recommended by Savell and Cross in 1988 to ensure consumer
63 acceptability in terms of eating quality. However, a more recent study in Australia recommended
64 a minimum of 5% IMF (Hopkins, Hegarty, Walker & Pethick, 2006) in lamb meat. The ability of
65 CT to predict IMF has been confirmed in a number of studies in live lambs of different breeds
66 (Young et al., 2001; Karamichou, Richardson, Nute, McLean & Bishop, 2006; Macfarlane,
67 Lewis, Emmans, Young & Simm, 2009; Navajas, Lambe, Bünger, Glasbey, Fisher, Wood et al.,
68 2006; Lambe, Navajas, Schofield, Fisher, Simm, Roehe et al., 2008; Clelland, Bunger, McLean,
69 Conington, Maltin, Knott et al., 2015) and in live pigs (Lambe, Wood, McLean, Walling,
70 Whitney, Jagger et al., 2013), providing a rare *in-vivo* predictor of meat quality. Most of these
71 studies found the strongest CT predictors of IMF to be average density values of the pixels in the
72 CT images associated with muscle and, in some cases, fat. Recently, the first genetic parameters
73 for CT-predicted IMF were estimated and moderate heritabilities reported (Clelland, Bunger,
74 McLean, Knott & Lambe, 2015). Muscle density measured by CT has also been shown to be
75 moderately correlated (genetically and phenotypically) with sensory eating quality traits
76 (Karamichou et al., 2006; Lambe, Navajas, Fisher, Simm, Roehe & Bunger, 2009). Therefore,
77 CT has the capability to simultaneously select sheep for higher eating quality (IMF) and lower
78 waste (carcass trim-able fat).

79
80 Computed tomography is non-invasive and non-destructive, so can be used on cuts of meat for
81 human consumption, which would reduce the costs and impracticalities associated with scanning
82 live animals. This could make selection for meat quality feasible, if suitable predictors can be
83 assessed in meat cuts and fed back to breeding programmes, or could be used to sort lamb cuts
84 into quality grades based on meat quality characteristics.

85

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

98

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

104

105 **2. Material and methods**

106 *2.1. Loin cut sourcing*

107 On each of three consecutive days in October 2014 (days 1, 2 and 3), approximately 100 lamb
108 carcasses were selected from the commercial slaughter line at Wm Morrison's Woodhead
109 Brothers abattoir in Turriff, Aberdeenshire. A total of 303 loins were collected. The aim was to
110 sample from all fat and conformation classes within the EUROP carcass classification grid, as
111 employed in the UK for carcass grading. Immediately post-slaughter, after dressing, hot
112 carcasses were subjectively classified by a trained grader into conformation classes E, U, R, O,
113 P (E being excellent conformation and P poor conformation) and fat classes 1 (very lean) to 5
114 (very fat), with fat classes 3 and 4 subdivided into low (L) and high (H). Fat class is expected to
115 correspond to the estimated subcutaneous fat percentage of the carcass: 1 = 4, 2 = 8, 3L = 11,
116 3H = 13, 4L = 15, 4H = 17 and 5 = 20 (Kempster, Cook & Grantley-Smith, 1986). The

117 distribution of carcasses in the dataset across these classes is shown in Table 1. No carcasses
 118 with conformation class P (poor) were available, but the distribution is believed to reflect the
 119 typical spread of carcasses through this abattoir during this prime slaughter period.

120

121 **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 |

122

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

133

134 2.2 Computed tomography scanning

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

139

140 The amount of absorption of x-rays during CT scanning depends on tissue density and can be
 141 quantified using CT numbers (relating to greyscale values in the resulting image) or Hounsfield
 142 units (HU). The CT scanner is calibrated to assign water a value of 0 HU and air a value of –
 143 1000 and different tissues can be assigned values ranging from –1000 to +1000 HU (Wegner,
 144 1993). Density values of each pixel can be denoted either as greyscale values or as Hounsfield
 145 units, after undergoing a linear transformation. Computed tomography images were segmented
 146 using a multi-object animal tomograph analysis routine (ATAR) software, developed at
 147 BioSS/SRUC (Mann et al., 2013). From the density value assigned to each pixel in each image
 148 by the CT scanner, pixels were allocated as fat, muscle, or bone, using previously-developed
 149 density thresholds, specific to the analysis of images obtained from carcasses, primal cuts and
 150 dissected muscles (Table 2). Tissue weights were calculated using area and density (converted to
 151 g/cm^3) values for each tissue. The CT density results for each tissue were then weighted by area
 152 in each image and averaged over the spiral series images (26-30 images per loin, average = 28
 153 images) to give an overall average density value (and its standard deviation) for fat and muscle.
 154 Additionally, the pixels allocated as fat and muscle were combined to give overall “soft tissue”
 155 weights and density values (Table 2).

156

157 **Table 2:** Tissue density thresholds (expressed in both greyscale and Hounsfield units) applied
 158 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 |

159

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

163

164 *2.3 Intramuscular fat extraction*

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

170
171 Samples were later thawed and fatty acid analysis was carried out by direct saponification as
172 described in detail by Teye et al. (Teye, Sheard, Whittington, Nute, Stewart & Wood, 2006).
173 Samples were hydrolysed with 2M KOH in water:methanol (1:1) and the fatty acids extracted
174 into petroleum spirit, methylated using diazomethane and analysed by gas liquid
175 chromatography. Samples were injected in the split mode, 70:1, on a CP Sil 88, 50m30.25mm
176 fatty acid methyl esters (FAME) column (Chrompack UK Ltd, London, UK) with helium as the
177 carrier gas. The output from the flame ionization detector was quantified using a computing
178 integrator (Spectra Physics 4270; Darmstadt, Germany) and linearity of the system was tested
179 using saturated (FAME4) and monounsaturated (FAME5) methyl ester quantitative standards
180 (Thames Restek UK Ltd, Windsor, UK). All measurements of fatty acids were performed in
181 duplicate, the error between replications being usually 1% to 2% with a maximum allowance of
182 5% error. Individual fatty acids were not considered for this paper, but will be the subject of
183 further analysis. Total IMF content was calculated gravimetrically as total weight of fatty acids
184 extracted, which was the trait of interest for the current study.

185
186 *2.4 Mechanical tenderness*
187 The thawed loin sample was cooked 'sous vide' in a polythene bag submerged in a temperature
188 controlled water bath (80°C). Core temperature of the loin was constantly monitored until an
189 internal core temperature of 75°C was reached. Loins were rapidly cooled and stored under
190 refrigeration for up to 24 hours. Loin samples were then prepared for shear force testing by
191 cutting ten 10mm x 10mm x 20mm core samples from each loin. Samples were sheared using a
192 MIRINZ tenderometer (bite test) to assess the force required to shear through the sample
193 (Bickerstaffe, Bekhit, Robertson, Roberts & Geesink, 2001). Average peak force (ShF) was
194 recorded as the mean peak force across a maximum of ten samples for each loin.

195

196 *2.5 Taste panel evaluation*

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

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

210

211 *2.6 Statistical analysis*

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

218

219 **Table 3:** Parameters tested in the GLM models to predict meat quality and meat eating quality
220 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 |

| | | | |
|----------|----------------------------------------------------------|------|-----------------------------------------------------------------------------------------------------------------------|
| MWT | Weight of muscle estimated by CT (g) | FSD | weighted by fat area (HU) 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 | | |

221

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

230

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

238

239 A second regression method, partial least squares regression (PLSR), was then used in Genstat
 240 (Payne *et al.*, 2013) to investigate whether more accurate predictions of meat quality could be
 241 made by considering the frequency distributions of pixel density values (on the greyscale).
 242 Numbers of pixels of each greyscale value (summed across all 2-dimensional CT images in the
 243 spiral scan series) were used as predictor variables (X) and IMF and other meat quality traits as

244 predicted variables (Y). PLSR is particularly suited when the matrix of predictors has more
245 variables than observations, and when there is multicollinearity among X values. PLSR considers
246 linear combinations of the independent variables as factors and adds successive factors to both
247 minimise the residuals and simultaneously to have high squared covariance with Y variables.
248 Multiple factors (or dimensions) are constrained to be mutually orthogonal. For this dataset,
249 cross-validation was performed (using 3 groups, split by slaughter day) in order to choose the
250 correct number of dimensions of histogram values to explain each meat quality trait without
251 over-fitting the PLSR equations. The optimal number of dimensions in each equation was
252 determined in Genstat using the predictive residual error sum of squares (PRESS) and Osten's F-
253 test, which tests the significance of incremental changes in PRESS (Osten, 1988).

254
255 To determine the relationships between IMF (or CT-predicted IMF) and sensory traits,
256 regression analyses (linear and polynomial to order 3) were performed in Genstat. To assess the
257 potential for consumers to differentiate between meat samples sorted into different IMF grades
258 or bands (either by chemical analysis or CT prediction equation), IMF values were also grouped
259 into bands and fitted as a factor in a regression model in Genstat, to explain variation in sensory
260 traits.

261

262 **3. Results**

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

270

271

272 **Insert Figures 1a and 1b here**

273

274 **Table 4:** Summary statistics for meat (eating) quality traits in the full data set, the calibration
 275 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 traits² | | | | | | | | |
| 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 |

276 ¹Number of IMF records, the number of records for other traits may vary by a maximum of 4

277 ²See Table 3 for trait descriptions and units

278

279 *3.1 Prediction of meat quality traits using CT*

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

285

286 **Table 5:** Correlations (r) between meat (eating) quality traits and model parameters. Values in
 287 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 |

288

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

293

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

304

305 **Table 6:** Prediction accuracy of meat (eating) quality traits using the best models in OLR,
 306 considering the full data set, calibration data set (slaughter days 1&2) and validation data set
 307 (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 |

308

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

310
$$\text{IMF}\% = 2.897 + (0.0797 \cdot \text{CCWT}) - (0.000692 \cdot \text{LoinWT}) + (0.02477 \cdot \text{FD}) + (0.029 \cdot \text{MD}) -$$

311
$$(0.0488 \cdot \text{STD})$$

312 Model 1

313

314 Using this prediction equation, the ability of CT to categorise lamb cuts into different IMF bands

315 was investigated, to assess its potential for sorting into different grades of meat quality. The

316 accuracy of prediction for the other meat quality traits was considered too low to make similar

317 analyses worthwhile for those traits. Categorising IMF into 5 percentage bands, the actual

318 chemical IMF band was matched to the predicted IMF band (when IMF was predicted using

319 Model 1). The frequency of samples assigned to each band is shown in Table 7. In total, 53.5%

320 of samples were correctly assigned to band (shown in bold, Table 7). The majority (63.3%) of

321 samples with chemical IMF < 3% were assigned a band below 3-4% by CT, however, 25.2% of

322 those with IMF > 3% were assigned as below band 3-4%. Of the samples with IMF > 4%

323 (n=29), only 1 sample was assigned as greater than band 3-4%. These results suggest that the CT

324 prediction equation is less good at identifying samples in the bands at the high end of the IMF

325 distribution.

326

327 **Table 7:** Frequency of samples in each IMF percentage band¹

| 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 |

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

330

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

335

336 **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 |

337

338 *3.2 Relationships between IMF and sensory traits*

339 Linear and polynomial regressions were tested between IMF and sensory traits (including
340 outliers). Fitting a quadratic regression accounted for the largest proportion of variation in
341 texture (R² = 8.6%) and overall liking (R² = 11%) (Figure 2), suggesting that these traits peaked
342 between 4 and 5% IMF. However, a linear regression gave the best fit between IMF and the
343 sensory traits of flavour (R² = 10%) and juiciness (R² = 5.7%) (Figure 2), implying that these
344 traits continued to rise with IMF across the range of IMF values observed.

345

346 **Insert Figure 2 here**

347

348 Regressing CT-predicted IMF on sensory scores, gave slightly lower accuracies of prediction
349 (7% for texture and flavour, 5% for juiciness and 8% for overall liking) and, for these
350 relationships, fitting non-linear trend lines did not improve fit over the linear relationships.

351

352 **3.2.1. Chemical IMF results.** To assess the ability of consumers to differentiate between meat
 353 samples assigned into “bands” of meat quality, IMF values were grouped into bands, as above,
 354 and the relationship with variation in sensory traits tested (Figure 3). Significant differences were
 355 observed in sensory traits between IMF bands. Samples in the IMF range 4-5% had the highest
 356 mean score for all taste panel traits, except juiciness, where samples with >5% IMF had the
 357 highest mean score. Samples with IMF of 4-5% scored significantly higher (P<0.05) than
 358 samples with <3% IMF for all traits except juiciness. For all traits except texture, samples with
 359 3-4% IMF scored significantly higher than samples with <3% IMF. Due to the small number of
 360 samples with >5% IMF, this category was not significantly different from any other IMF band
 361 for any of the sensory traits. Samples in the 3-4% IMF band were not significantly different to
 362 those in the 4-5% band for any sensory trait.

363

364 **Insert Figure 3 here**

365

366 **3.2.2. CT-predicted IMF results** To assess the ability of consumers to differentiate between
 367 meat samples assigned by CT into “bands” of meat quality, CT-predicted IMF values (using
 368 Model 1) were grouped into bands. Values fell into four bands, but there were only 8 records in
 369 the 1-2% band and only 2 records in the 4-5% band, so these were incorporated into the other
 370 bands, which were then defined as <3% or ≥3% CT-predicted IMF. The results of the GLM
 371 model fitting these bands to explain variation in sensory traits are shown in Table 9.

372

373 **Table 9:** Predicted means for meat (eating) quality traits at each CT-predicted IMF band level¹

| | Adj- R ² | CT-predicted IMF band | | P value |
|-----------|------------------------|-----------------------|------|---------|
| | | <3% | ≥3% | |
| 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 |

374

375

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

381

382 **4. Discussion**

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

401

402 Previous studies in beef and pork have used a partial least squares regression (PLSR) approach to
403 estimate IMF from CT data (Prieto et al., 2010; Furnols et al., 2013; Kongsro & Gjerlaug-Enger,
404 2013). In the work by Furnols et al. (2013), OLR, using 3-6 independent variables describing
405 relative volumes relating to density ranges, was found to predict IMF in pork loins with similar
406 or greater accuracy than PLSR that used many more variables, especially where IMF levels were

407 low. However, both types of models resulted in higher prediction accuracies ($R^2 = 0.63-0.83$)
408 than have been achieved in the current study on lamb. In a study by Kongsro et al. (2013), PLSR
409 was not considered a feasible method to predict IMF in pig loins *in vivo*, due to low prediction
410 accuracies and high prediction errors, although limited variation in IMF in the sample population
411 is likely to have contributed to these results. The PLSR approach was also previously considered
412 for predicting IMF from CT in live lambs (Lambe, Jopson, Navajas, McLean, Johnson &
413 Bunger, 2009), but was found to be less transportable between data sets/ populations than other
414 prediction equations derived by OLR methods. Furthermore, it is more difficult to assign
415 biological meaning to PLSR results. The prediction equation derived by the OLR method in the
416 current study (Model 1) gave the best prediction of IMF using CT data from lamb primal cuts,
417 but is unlikely to be accurate enough to be used on its own as a means of sorting meat cuts into
418 quality grades or within breeding programmes to select for meat quality.

419

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

432

433 The CT parameters measured in the vacuum-packaged loin cuts were poor predictors of ShF and
434 sensory traits. Low prediction accuracies (R^2 0.03 – 0.14) were previously reported (Clelland,
435 2015) for mechanical shear force using a range of CT parameters measured in live lambs, across
436 different lamb breeds. Similarly, low correlations ($< \pm 0.3$) were observed between CT muscle
437 density, measured *in vivo*, and shear force or taste panel sensory scores, using loin or leg muscles

438 from two divergent lamb breeds, whilst these density measurements had moderate to high
439 negative correlations (-0.3 to -0.7) with IMF (Lambe et al., 2008). These results are also
440 consistent with findings from a study of Scottish Blackface lambs (Karamichou et al., 2006),
441 where low phenotypic correlations (-0.16 to -0.29) were estimated between shear force or taste
442 panel sensory traits and CT muscle density. However, that study was powerful enough to
443 estimate genetic correlations with CT muscle density, which were found to be considerably
444 stronger (-0.49 to -0.8), for all traits except texture assessed by taste panel.

445
446 As in other livestock species, IMF in lamb has been shown to have positive effects on consumer
447 sensory scores of tenderness, juiciness, flavour and overall liking (Pannier, Gardner, Pearce,
448 McDonagh, Ball, Jacob et al., 2014). In the current study, IMF accounted for 11% of the
449 variation in overall liking, as assessed by a trained taste panel, and between 5-10% in the other
450 sensory traits. Interestingly, the effects of IMF on texture and overall liking appeared to be curvi-
451 linear, with an optimum IMF level between 4 and 5%, whereas linear positive relationships were
452 observed between IMF and juiciness or flavour, in the lamb samples studied. However, there
453 were low numbers of samples with high IMF levels within the sample population, and few
454 samples with values higher than 5% IMF, so this could not be fully tested and should be
455 interpreted with caution. It would be of interest to confirm these findings using samples
456 representing a greater range of IMF values. Only 3% of the variation in overall liking was
457 explained by IMF in the Australian study by Hopkins et al. (2006), using an untrained consumer
458 panel, where the samples spanned a much wider range of IMF values (2-18%), with a higher
459 mean value of 5.4%. A review of literature to the late 1980s by Savell and Cross (1988)
460 recommended a minimum of 3% IMF for grilled cuts of lamb, such as the loin, to ensure
461 consumer acceptability. A decade later, a study of German Longwool Merino lambs (Heylen,
462 Suess, Freudenreich & von Lengerken, 1998) found that scores of sensory properties increased
463 with IMF in the loin, with IMF content greater than 2.3% resulting in profound improvements in
464 sensory traits, especially flavour and overall acceptance. That study found that sensory
465 characteristics in the LL scored most highly in meat with between 3.5 and 4.5% IMF. However,
466 a minimum of 5% IMF in lamb was recommended based on Australian consumer preferences
467 (Hopkins et al., 2006). These differences may be partially due to experimental design, as well as
468 differences in consumer preferences between countries or across time. The current results,

469 suggesting a peak in overall liking between 4 and 5% IMF, appear to agree with the earlier
470 studies, rather than the Australian study, suggesting geographical differences in preference rather
471 than any consistent changes in consumer preference across recent decades.

472
473 Further analysis of the taste panel results (not presented here) suggested that the main sensory
474 trait affecting overall liking was flavour, which accounted for 79% of variation in overall liking
475 when fitted in a simple linear regression, compared to texture, which accounted for 20% and
476 juiciness, which accounted for 14%. No information was known about the breed, age or feeding
477 regime of the lambs that were sampled, all of which are known to affect lamb flavour (Duckett
478 & Kuber, 2001). Greater standardisation of these factors may have made it easier to identify the
479 role of IMF and ShF in overall liking. The specific compounds responsible for lamb flavour have
480 yet to be determined, but fatty acid profiles may play a role (Duckett & Kuber, 2001). The
481 current study collected information on individual fatty acid composition within the samples, the
482 analysis of which was considered outside the remit of this paper and will be the subject of a
483 future study, which could provide some useful insights into factors affecting palatability.
484 Differences in fatty acid composition could also affect fat density, and this could differ
485 depending on storage time and conditions, which could alter some of the CT parameters studied.
486 These factors may be influencing results in the present study and more work is needed in this
487 area to understand these effects.

488
489 Given that IMF predicted around 11% or less of the variation in taste panel traits in the lamb
490 samples studied here, and CT parameters directly predicted 10% of the variation in overall
491 liking, the question arises whether we should try to increase accuracies to be able to predict IMF
492 with CT (accuracies currently ~36%) in an effort to improve meat eating quality, or directly
493 predict taste panel (sensory) traits? The sensory traits have lower prediction accuracies, but are
494 the ultimate goal for consumer acceptability. However, scoring of sensory traits is subjective,
495 whereas IMF measurements are objectively measured and should be repeatable. Work in live
496 lambs also suggests that prediction accuracies for IMF using CT could be increased by scanning
497 multiple sections or whole carcasses. The sensory scores in the current study were assessed by a
498 trained taste panel, rather than a consumer taste panel, so may be more consistent, leading to
499 higher prediction accuracies of these traits than have been found in other studies. Selection on

500 predicted values of an objective trait, such as IMF, is likely to be more successful in identifying a
501 consistent product that can be tailored to different markets. Additional samples have been stored
502 from these loins and will be used in future, alongside the lab and taste panel results, to design
503 and conduct a consumer taste panel. This will further assess some of these relationships.

504

505 **5. Conclusions**

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

518

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525

526

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