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Genotypic effects of the Texel Muscling QTL (TM-QTL) on meat quality in purebred Texel lambs

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ABSTRACT

Texel Muscling QTL (TM-QTL) increases loin muscling in lambs inheriting it from their sire only. This study investigated TM-QTL effects on meat quality in 209 Texel lambs that were CT-scanned then slaughtered at 20 weeks (carcasses aged for ~1 week). Loin meat quality traits included: CT-measured muscle density (predicting intramuscular fat); mechanical tenderness using Volodkevich-type jaws or MIRINZ tenderometer; intramuscular fat; sensory eating quality (sub-sample of 40 lambs). Volodkevich tenderness was also measured in the leg (*Vastis lateralis*). TM-QTL genotypes were determined, giving 40 non-carriers (+/+), 70 heterozygotes—53 inheriting TM-QTL from the sire (TM/+) and 17 from the dam (+/TM), 34 homozygote TM-QTL lambs (TM/TM) and 65 uncertain. Multiple regression identified no genotype effects on meat quality. For MIRINZ-measured loin tenderness only, contrasts revealed a significant additive effect of TM-QTL (1.27 kgF difference between homozygotes). However, the taste panel identified no significant differences between +/+ and TM/TM lambs. Results show little evidence of TM-QTL affecting meat quality.

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1. Introduction

The Texel Muscling quantitative trait locus (TM-QTL), which was identified on chromosome 18 (OAR18) in Texel sheep (Walling et al., 2004), has been found to increase loin muscle dimensions and weights (by ~4 to 14%) in purebred and crossbred carrier lambs (Macfarlane et al., 2009, 2010; Walling et al., 2004). In a previous study, Macfarlane et al. (2010), using the same purebred Texel lambs as those used in this study, reported that TM-QTL appeared to be paternally imprinted exhibiting a polar overdominant mode of inheritance. Direct additive effects for loin muscling, measured by ultrasound, CT scanning or dissection, were only expressed if the lamb inherited one copy of the TM-QTL allele from its sire. Depending on trait and measurement method, loin muscling increased by 4 to 11% compared to non-carriers.

Other mutations reported to be in close proximity to the TM-QTL on OAR18 are the Callipyge mutation (*CLPG*) (Cockett et al., 1996) and the rib-eye muscling QTL, also known as the Carwell locus (Nicoll et al., 1998). The *CLPG* mutation is associated with greatly increased muscularity, mainly in lumbar and pelvic regions, and reduced carcass fat (Cockett et al., 1996, 1999). This mutation has also been found to

exhibit a polar overdominant mode of inheritance, with paternally-expressed imprinting (Cockett et al., 1996; Freking et al., 1998). However, *CLPG* lambs have substantially tougher loin meat, even after 24 days conditioning post-slaughter (e.g. Duckett, Klein, Dodson, & Snowden, 1998). The rib-eye muscling QTL shows a smaller phenotypic effect than *CLPG*, similar to that of TM-QTL (Masri et al., 2010). The rib-eye muscling QTL results in significantly tougher loin meat in heterozygous carriers than in non-carriers (Jopson et al., 2001), however, this effect was not significant after enhanced post-slaughter processing (chilling for six weeks prior to testing). Compared to samples from non-carrier lambs, loin muscle samples from crossbred (Texel × Mule) TM-QTL carrier lambs, inheriting the QTL from their sire, were found to have increased toughness, although there was no significant QTL effect on leg muscle tenderness (Lambe, Haresign, et al., 2010; Lambe, Macfarlane, et al., 2010). The significant effect of TM-QTL on loin toughness was observed using two different mechanical tenderness tests: Volodkevich and MIRINZ (Lambe, Macfarlane, et al., 2010). However, the effects on loin tenderness were not shown after extended conditioning of the meat for 9 days (Lambe, Haresign, et al., 2010). Intramuscular fat (IMF) in the loin muscle was also found to be significantly lower in male crossbred TM-QTL carrier lambs than in male non-carriers (Lambe, Macfarlane, et al., 2010). Lower levels of IMF could negatively affect meat quality of slaughter lambs inheriting this QTL, especially given that IMF levels in loin muscle from this crossbred are already lower than recommended

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levels to ensure consumer acceptability (Lambe, Macfarlane, et al., 2010; Savell & Cross, 1988).

Although a polar overdominant mode of inheritance, with paternally-expressed imprinting, has been identified for the direct effects of TM-QTL on loin muscling (Macfarlane et al., 2010), the effects on meat quality traits of inheriting this QTL from either or both parents still requires further investigation. The models reported for effects of the *CLPG* mutation on carcass traits (eye muscle area, rump width, leg score, carcass lean) included a polar overdominant mode of gene action, with no additive or maternal dominance effects (Freking et al., 1998). However, for shear force, calpastatin activity and marbling score, significant additive effects were identified, as well as paternal polar overdominance effects, whilst further maternal dominance effects were also significant for some traits (shear force and calpastatin activity at day 0, when adjusted for carcass weight) (Freking et al., 1999).

The aim of this study, therefore, was to investigate the effects of the mode of inheritance of the TM-QTL on Texel lamb meat quality (tenderness and intramuscular fat content).

2. Material and methods

2.1. Live animal measurements

A total of 209 purebred Texel lambs (114 females, 95 entire males) were raised at pasture on two different experimental research farms: one in Wales and one in Scotland. Lambs were sired by seven different rams (three common across farms) that were carriers of TM-QTL. Some of the ewes in each flock also carried TM-QTL. The majority of lambs ($n = 140$) were reared as singletons, 57 as twins and 12 were artificially reared. Lambs were blood-sampled for genotyping and were reared to approximately 20 weeks of age, when they were ultrasonically scanned to measure muscle and fat depths over the third lumbar vertebra. Lambs were also CT scanned and average muscle density (CT_MD) was measured in a cross-sectional scan taken at the 5th lumbar vertebra. CT_MD has been shown to be a good predictor of intramuscular fat content and is related to meat eating quality (Karamichou, Richardson, Nute, McLean, & Bishop, 2006; Macfarlane, Young, Lewis, Emmans, & Simm, 2005; Young, Simm, & Glasbey, 2001). The majority of lambs were CT scanned the week before slaughter (average age 133 days). However, 40 lambs were scanned 3 weeks earlier (average age 114 days) to allow them to undergo taste panel analyses after the 30-day withdrawal period from the sedative used during the CT process. All procedures involving animals were approved by the SAC animal ethics committee and were performed under UK Home Office licence, following the regulations of the Animals (Scientific Procedures) Act 1986.

2.2. Carcass and meat quality measurements

Mean age at slaughter was 144 days (s.d. 7.5, range 126–155 days) and mean hot carcass weight was 15.2 kg (s.d. 3.1, range 8–25 kg). Carcasses underwent high voltage electrical stimulation. After chilling for between 7 and 9 days, carcasses were dissected and muscles from the loin (*M. longissimus lumborum*) and leg (*M. vastus lateralis*) were vacuum-packed and frozen.

Muscle samples from the right carcass side were transported to the University of Bristol for shear force and intramuscular fat assays. Following thawing, toughness was measured in both muscles, after cooking (in vacuum pack bags) in water at 80 °C to an internal temperature of 78 °C (Teye et al., 2006). Samples were cooled in ice and then held at 4 °C. Ten 10 × 10 × 20 mm blocks were cut from each muscle in the direction of the muscle fibres and sheared, using a TA-XT2 texture analyser (Stable Micro System, Surrey, UK) fitted with Volodkevich-type jaws. Toughness (ToughA) was recorded as the force (kgF) required to compress the sample, with higher values for

tougher (less tender) samples. Results were averaged from the 10 sub-samples. Intramuscular fat percentage (IMF) was also measured in the loin muscle using petroleum ether (B.P. 40–60 °C) as the solvent in a modified Soxhlet extraction.

A second mechanical tenderness test (ToughB), was conducted at SAC Edinburgh, using a MIRINZ tenderometer (<http://www.agresearch.co.nz/mirin/docs/meat-science.pdf>) on 169 loins. This test uses a similar method to ToughA, but slightly different compression equipment, to quantify toughness. For practical reasons, the samples were removed from the freezer between 1 and 3 days before testing, and the length of this period was recorded to allow later adjustments in the statistical model. For this test, samples were cooked in a 100 °C water bath to an internal temperature of 75 °C (Bickerstaffe, Bekhit, Robertson, Roberts, & Geesink, 2001). Cooked samples were then chilled and held at 2 °C for 48 h before testing. Summary data for each meat quality trait measured is presented in Table 1.

Both ToughA and ToughB are examples of mechanical tenderness tests that use compression of the muscle sample, rather than shearing. Previous work has suggested that compression tests may be more indicative of the effect of connective tissue content on tenderness, whereas shearing is more closely associated with myofibrillar structure and its effects on tenderness (Lepetit, Salé, & Ouali, 1986). The Volodkevich compression test attempts to imitate the incisor biting action and compresses samples of cooked meat between two opposing rounded blades (Volodkevich, 1938). The MIRINZ test (Bickerstaffe et al., 2001) conducts a similar compression test, but using a profiled tooth against a 3 mm diameter rod.

Loin muscles from the left carcass side of the sub-set of animals selected for taste panel analyses ($n = 40$; all of which had been aged on the bone for 7 days prior to freezing) were transported for sensory evaluation (details below) to Faccenda in Brackley, a privately-owned company specialising in the supply of fresh poultry to the UK market.

2.3. Sensory evaluations

A consumer sensory panel of fourteen assessors was used to assess different eating quality attributes of the 40 lamb loins. Although run as an untrained panel, some individuals had prior experience of assessing poultry meat in trained taste panels. Loin samples were thawed and brought to room temperature immediately before cooking. All the loin samples were cooked at the same time in the same industrial oven to an internal temperature of 72 °C and held for 2 min at this temperature, then removed from the oven and allowed to rest for 5 min. Panellists were instructed to taste in silence and to clear the palate with water or water biscuits between each sample, and note their scores on a standard taste panel record sheet. Each assessor scored between 4 and 14 samples from different animals (mean and median of 7.5). The number of assessors scoring a sample

Table 1

Summary of data from lambs of known genotypes available for each objective and sensory^a meat quality trait.

	Number of records per genotype				Mean	s.d.	Min	Max
	+/+	+/TM	TM/+	TM/TM				
ToughA_loin (kgF)	40	17	53	34	3.13	1.30	1.77	9.83
ToughA_leg (kgF)	40	17	53	34	2.89	0.53	1.77	4.95
ToughB_loin (kgF)	27	17	53	16	3.78	1.48	1.84	9.44
IMF (%)	40	16	52	33	1.34	0.54	0.37	2.87
CT_MD (HU) ^b	39	17	53	34	46.2	3.5	36.5	55.9
Appearance ^a	13	0	0	18	6.38	1.25	4.00	9.00
Flavour ^a	13	0	0	18	6.53	1.12	3.50	9.00
Texture ^a	13	0	0	18	6.20	1.30	2.50	8.33
Succulence ^a	13	0	0	18	6.29	1.17	4.00	9.00

^a Summary statistics relate to average values for each lamb across assessors (from 1 = very poor to 10 = excellent).

^b Hounsfield units (HU = $(D - 1.0062)/0.00106$, with density [D] in g/cm³).

from the same animal varied between 1 and 5 (mean = 2.625 and median = 3) (Fig. 1). Eight loins were only scored by a single assessor. Assessors scored samples for appearance, flavour, texture and succulence, each on a 1–10 scale (Table 1), with a score of 1 relating to very poor and 10 relating to excellent.

2.4. Lamb genotyping

For each lamb, the TM-QTL status was determined based on genotypes at 5 microsatellite markers on ovine chromosome 18, which were genotyped by Pfizer Animal Genetics, New Zealand. Within-family linkage phase between marker haplotypes and QTL status were estimated from the data and TM-QTL genotypes inherited from the sire and dam were determined accordingly, as described in detail by Macfarlane et al. (2010). For some lambs, the TM-QTL genotypes inherited from one or both parents were unknown (due to recombinant haplotypes, missing marker information, or unknown parentage). The final number of lambs with informative genotypes included 40 non-carrier lambs (+/+), 53 heterozygote carriers with TM-QTL inherited from the sire (TM/+), 17 heterozygote carriers with TM-QTL inherited from the dam (+/TM) and 34 homozygote TM-QTL lambs (TM/TM). Only the results for these genotypes were included in the main analyses and are presented. One lamb was missing CT_MD data, whilst three were missing IMF data (Table 1).

The taste panel samples were selected, following preliminary genotyping analyses, to provide equal numbers of lambs that were expected to be +/+ or TM/TM genotypes. These samples were selected before the polar overdominant model of inheritance was detected by Macfarlane et al. (2010), with the expectation that the two homozygous groups would be most divergent in muscling phenotype. However, after the final genotyping analyses had been performed, including phenotypic ultrasound data from all lambs pre-slaughter, the final number of taste panel lambs in each genotype class was 13 +/+ and 18 TM/TM, with 9 lambs with genotyping inconclusive. Samples from unknown genotypes were excluded from further analyses.

2.5. Statistical analyses

Multiple linear regression analyses were performed in Genstat (version 11 or later; Payne, Murray, Harding, Baird, & Soutar, 2008), to estimate the effects of TM-QTL genotype on:

- Tough A_loin: shear force in the loin muscle
- Tough A_leg: shear force in the leg muscle
- Tough B_loin: shear force in the loin muscle
- IMF_loin: intramuscular fat percentage of the loin muscle

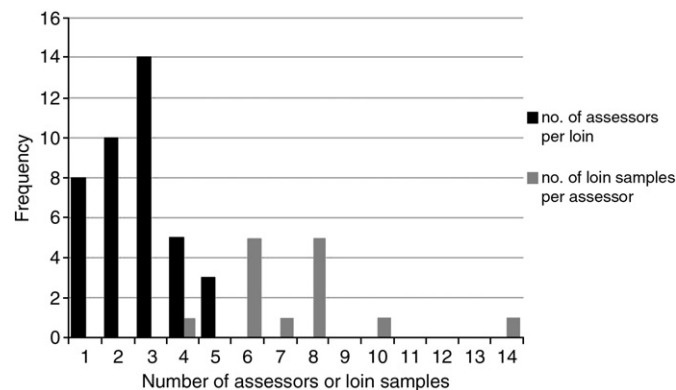


Fig. 1. Histogram of number of assessors scoring each loin and number of loins scored by each assessor.

- CT_MD: CT-measured average muscle density in a two-dimensional scan at the site of the 5th lumbar vertebra in the live lamb, measured in Hounsfield units (HU)

The model for each trait included fixed effects of sex, farm and litter size at rearing, where significant. Interactions amongst these three factors, or between sex and TM-QTL genotype, did not have a significant effect at the 5% level on any trait, so were not included in the models. Carcass weight was fitted as a covariate for post-mortem traits (slaughter age was tested in place of weight, but was not significant), whereas live weight was fitted as the covariate for CT_MD (age at CT was tested as an alternative, but was not significant). Days conditioning (7, 8, or 9) was also fitted as a covariate for the ToughA_loin measurements, but was not significant for ToughA_loin, ToughB_loin, or IMF. For ToughB_loin, the number of days between removing the sample from the freezer and measurement (1, 2, or 3) was tested as an additional covariate, but was not significant in the final model. Sire was tested as a random effect for each trait, as part of a mixed model, but only had a significant effect on CT_MD, so only fixed effects models were fitted for the other traits. The term significance here and throughout the paper refers to statistical significance at the 5% significance level.

Plotting fitted values against residual values revealed that the dispersion of the residuals increased as values increased for both ToughA_loin and ToughB_loin. Therefore, logarithmic transformations (natural logs) were performed on these two traits prior to analyses. For the other traits, no transformations were required and measurements were analysed on the original scales.

Three orthogonal contrasts were evaluated for each objective meat quality trait, to partition variation due to TM-QTL genotypic effects, after adjusting for all other effects in the model. Similar contrasts were fitted to +/+, +/TM, TM/+, TM/TM genotypes as those identified by Freking et al. (1998) to test for additive (−1, 0, 0, 1), dominance (−1, 1, 1, −1) and reciprocal heterozygote (0, −1, 1, 0) models of gene action. If a difference between heterozygotes was found, a further set of orthogonal contrasts could then be fitted to test for paternal polar overdominance (Freking et al., 1998; Freking et al., 1999), since this mode of inheritance had been identified for the direct effects of TM-QTL on loin muscling (Macfarlane et al., 2010), fitting a further set of contrasts to identify additive (−1, 0, 0, 1), maternal dominance (−1, 2, 0, −1) and paternal polar overdominance (−1, −1, 3, −1) effects.

For each sensory attribute, data were analysed as a mixed model using the technique of restricted maximum likelihood (REML). Genotype and order of presentation were fitted as fixed effects, while assessor was fitted as a random effect and constrained to have a non-negative variance component. As animals were randomly selected from the expected genotype populations, samples were treated as random effects rather than as fixed effects. Four alternative random models, all including assessor as a random effect, were fitted. This was in order to assess whether a separate variance component for samples should be fitted for each genotype or a common component across both genotypes, and also whether a separate residual variance component should be fitted for each genotype or a common residual variance component. Random model selection was based on changes at the 5% significance level in the deviance between nested models. For the effect of presentation order to the assessors (i.e. first sample presented, second sample presented ...) it should be noted that only two assessors scored ten or more samples. Hence there was insufficient data to include a separate factor level for all fourteen presentation positions. Instead, some grouping of positions was adopted. Thus, positions 7 and 8 were grouped together, as were positions 9 to 14. Presentation order was retained in the models irrespective of statistical significance, as such effects are known *a priori* to be a widespread phenomenon in sensory profiling.

Predicted means were obtained for samples. These are means adjusted for assessor and presentation order effects. As samples have been treated

as random effects, the predicted means for samples are shrunken towards the overall mean. It should be noted that the analysis assumes attributes were scored on an interval scale rather than simply on an ordinal scale. Thus equal increments on the scoring scale are assumed to correspond to equal increments in the sensory attribute; e.g. a change from 2 to 3 corresponds to the same improvement in flavour as from 7 to 8.

In order to examine the effects of TM-QTL genotype on variation of meat quality traits, genotype variances for traits analysed by regression were compared using Bartlett's test on the unstandardised residuals. For CT_MD, genotype variances were estimated as part of the mixed modelling and significance assessed by change in deviance from a model with a common variance.

3. Results

3.1. Objective tests of toughness and IMF

In contrast to earlier results found for direct effects of TM-QTL on loin muscling traits (Macfarlane et al., 2010), genotype did not significantly affect any of the objective meat quality traits studied, when adjusted for the model terms described above ($P > 0.05$; Table 2).

The percentage of variance accounted for by TM-QTL effects (additive + dominance + reciprocal heterozygote; 3 degrees of freedom), after adjusting for the other terms in the model (TM-QTL sums of squares as a percentage of corrected sum of squares) was less than 5% for all traits (Table 3).

There was a significant positive additive effect of TM-QTL on ToughB_loin (Table 3). This is in contrast to the results from the other traits and implies that, with the MIRINZ tenderometer measurement method, homozygous TM-QTL carriers had significantly tougher loin muscle than non-carriers. The reciprocal heterozygote effect was not significant for any trait, therefore the second set of contrasts (to test for paternal polar overdominance) were not applied.

After adjusting for model effects, variation was not statistically significantly different across genotypes for any trait, except for ToughA_leg, where variance in the TM/TM group was significantly lower than in the +/+ or TM/+ groups Table 4. Allowing for different variances for the genotypes for ToughA_leg in the regression analyses did not alter the conclusions presented in the previous tables (results very similar, so are not shown).

3.2. Subjective tests of sensory attributes

The results for the comparisons of genotype means of sensory attributes scored by the taste panel are summarised in Table 5. All means were in the acceptable to good range (scores 5 to 7). It should be noted that the predicted genotype means are derived from the loin (lamb) means and that equal weighting has been given to each lamb in deriving the genotype means. P-values for the presentation order

effect are for adding order of presentation into the model after genotype (i.e. after adjusting for differences attributable to genotype). Conversely, P-values for genotype are shown after adjusting for presentation order effects.

Statistical evidence at the 5% significance level for presentation order effects was found for appearance and succulence. However, in the case of the appearance attribute, the absence of a consistent trend in the corresponding means suggests the statistical significance of the presentation order effect may be spurious. Although predicted genotype means were higher (more desirable) for the carrier genotype than the non-carrier for all attributes (Table 5), none of these reached formal statistical significance using this small data set.

The variance components for variation between samples within each genotype and the residual variation (scorer \times sample interaction) within each genotype are shown in Table 6. This is in order to assess comparative consistency within genotypes. There was evidence of greater sample-to-sample variation in succulence for the carrier genotype than the non-carrier genotype, as shown by the "samples within genotype" variance components in Table 6. However, the within-genotype residual variation was less for the carrier genotype than for the non-carrier genotype in the case of succulence. This indicates greater consistency between assessors in scoring samples for the carrier genotype than for the non-carrier genotype. There was no statistical evidence of differences in either variance component between genotypes for appearance, flavour or texture attributes.

3.3. Relationships amongst objective and sensory meat quality measurements

Correlations between unadjusted data for objective techniques for predicting tenderness or IMF and average sensory trait scores awarded to each lamb by the taste panel are presented in Table 7. All lambs ($n = 209$) were included in this analysis, regardless of TM-QTL genotype. These results suggest that ToughA and ToughB measurements of the loin muscle, from opposite carcass sides, agree well ($r = 0.59$), and these loin toughness measurements have similar (moderately negative) correlations with IMF. However, correlations between loin toughness traits and ToughA_leg were lower ($r \sim 0.2$).

Considering correlations between objective and subjective measures of meat quality, ToughA_loin was moderately negatively correlated with texture score. The correlation between IMF and appearance was positive and moderate in magnitude. Flavour was significantly associated with increased IMF and reduced muscle density as measured by CT. Relationships between ToughA_leg and sensory traits were less relevant, since two different muscles were being tested, and correlations between ToughB and sensory traits could not be investigated, since loin muscles from the left carcass side were either used for one test or the other.

4. Discussion

4.1. TM-QTL effects on objectively-measured meat quality traits

The comparison of least-squares means found no significant evidence that there would be a negative effect on meat quality from introducing TM-QTL into a Texel population where the QTL was not already present, since genotype effects were non-significant on each trait studied. However, the results from the orthogonal contrasts suggest that ToughB_loin measurements in lambs inheriting two copies of TM-QTL show greater toughness than non-carrier lambs. These results are unlike previous results for the CLPG mutation.

Freking et al. (1999) found evidence of significant effects (additive, maternal dominance and paternal polar overdominance effects) on shear force adjusted for carcass weight and the CLPG mutation accounted for over 40% of the variation in these and other meat quality traits. The effects of the TM-QTL on the meat quality traits

Table 2
TM-QTL genotype least-squares means, average standard errors of difference (s.e.d.) and significance values (P -value) for each meat quality trait.

	+/+	+/TM	TM/+	TM/TM	s.e.d.	P -value
ToughA_loin (kgF) ^a	1.09 (2.97)	1.04 (2.82)	1.11 (3.03)	1.03 (2.79)	0.07	0.58
ToughB_loin (kgF) ^a	1.16 (3.18)	1.29 (3.65)	1.28 (3.59)	1.40 (4.04)	0.09	0.09
ToughA_leg (kgF)	2.92	2.76	2.91	2.87	0.13	0.71
IMF (%)	1.36	1.32	1.32	1.36	0.11	0.94
CT_MD (HU) ^b	45.05	45.39	45.88	45.23	0.62	0.34

^a Least-squares means presented as log values (back-transformed values in parenthesis).

^b Hounsfield units (HU = $(D - 1.0062)/0.00106$, with density [D] in g/cm^3).

Table 3

Percentage of variation in meat quality traits due to TM-QTL genotype, estimates of contrasts, and significance levels (*P*-value) for additive, dominance and reciprocal heterozygote effects.

		ToughA_loin ^a	ToughB_loin ^a	ToughA_leg	IMF	CT_MD
% variance		1.0	4.5	0.8	0.2	0.8
Additive	Contrast (s.e.)	−0.060 (0.067)	0.239 (0.096)	−0.055 (0.119)	0.006 (0.101)	0.185 (0.608)
	<i>P</i> -value	0.38	0.01*	0.64	0.95	0.76
Dominance	Contrast (s.e.)	0.031 (0.105)	0.021 (0.133)	−0.122 (0.186)	−0.085 (0.156)	0.996 (0.838)
	<i>P</i> -value	0.77	0.88	0.51	0.59	0.24
Reciprocal heterozygote	Contrast (s.e.)	0.071 (0.081)	−0.014 (0.086)	0.145 (0.142)	−0.008 (0.120)	0.488 (0.679)
	<i>P</i> -value	0.38	0.87	0.31	0.95	0.47

^aContrasts expressed in log values.

**P*<0.05.

measured in our study were much smaller, explaining a maximum of 4.5% of the variation. However, this is in line with the lower direct effects (4 to 11%) of the TM-QTL on loin dimensions and weights (Macfarlane et al., 2010), as opposed to the 30% increases in the *longissimus* weights and dimensions (Cockett et al., 1999; Koohmaraie, Shackelford, Wheeler, Lonergan, & Doumit, 1995) due to the *CLPG* mutation. Moreover, the *CLPG* mutation was not found to be segregating in the Texel sire families initially used to identify the TM-QTL and was therefore not considered to be the causal mutation for TM-QTL (Bishop, pers. comm.).

A power calculation carried out prior to the study, based on variation in mechanical tenderness found in previous investigations, suggested that 40 lambs per treatment would be sufficient to identify biologically meaningful genotypic differences in ToughA. Despite efforts over several years to propagate sufficient Texel lambs of each genotype for this study, the difficulties in obtaining unambiguous genotypes for these lambs resulted in numbers in some of the genotype groups that were lower than this recommended number. However, for all objective tests apart from ToughB, numbers in the TM/+ and +/+ groups were at or above this threshold, suggesting that the comparisons between these genotypes, at least, are statistically robust. No significant differences were identified between these groups for any traits. This is an important comparison, since significant direct effects of TM-QTL on loin muscling were observed between these two genotypic groups. The width of the back-transformed 95% confidence interval for the difference in mean ToughA_loin values between TM/+ and +/+ groups was 0.42 kgF. Previous research at the University of Bristol (A.V. Fisher, pers. comm.) found that a taste panel difference of half a score unit in an eight point category scale for tenderness equated to approximately 0.85 kgF. An effect of the size found here is unlikely to be detected by consumers, although the data available is not able to confirm this.

For ToughB, some genotypic group sizes were smaller than for the other objective traits (Table 1). Retrospectively using the standard deviation found in the current experiment for ToughB (1.48) in a power calculation, indicates that 48 lambs would be required in each genotypic group to identify biologically meaningful differences at the 5% significance level using this test. Only the TM/+ group contained

this number of lambs, although the results from the contrasts found a significant additive effect of TM-QTL on this trait, suggesting significantly greater values for ToughB in the TM/TM lambs than in the +/+ lambs. The lower sample sizes used to test this trait may make these results less precise. However, the differences in results between ToughA and ToughB are not simply due to sampling, since similar results as those presented here were observed for ToughA_loin when using only samples from the same lambs that were tested for ToughB_loin (data not shown).

It is interesting to note that we previously used the same mechanical tests to determine the effects of TM-QTL on tenderness of different cross-bred lamb samples (Lambe, Macfarlane, et al., 2010) where the results from these two tests were complementary. The correlation between results from the two tests on the loin muscle in the current study is also reasonably high ($r=0.59$), although this is not borne by genotypic effects on ToughA and ToughB, which are not consistent. The recommended protocols for each device advocate slightly different internal cooking temperatures, with the samples measured with the MIRINZ test cooked at a higher temperature (100 vs. 80 °C) to obtain to a slightly lower internal temperature (75 vs. 78 °C) than those measured by the Volodkevich test. The effect of connective tissue is more likely to be detected at lower temperatures (Peachey, Purchas, & Duizer, 2002), but it is unlikely that this 3° difference is sufficient to identify differences observed in our case, particularly since the *longissimus* muscle contains little connective tissue. Although further tests to explain some of the structural or chemical reasons for these differences in tenderness (e.g. sarcomere length, histology) are envisaged, they were not part of the current study, making it difficult to understand the reason for these differences at this stage.

As discussed in our previous work (Lambe, Haresign, et al., 2010), it is not straightforward to establish an agreed upper threshold value for mechanical tenderness, which corresponds to a reduction in consumer acceptability, because of the differences in technique, equipment, muscles and species used in past experiments. However, other studies on tenderness of lamb and beef suggest that a shear force threshold of around 5 to 5.5 kgF as an the upper value for a consumer acceptance window may be a reasonable assumption, if using the Warner-Bratzler technique (Destefanis, Brugiapaglia, Barge, & Dal Molin, 2008; Miller, Carr, Ramsey, Crockett, & Hoover, 2001; Platter et al., 2003; Shorthose, Powell, & Harris, 1986), whilst for the MIRINZ tenderometer thresholds of 8–11 kgF have been suggested

Table 4

Variances relating to residual data for each objective meat quality trait within each genotype and significance of differences between variances (*P*-value).

	+/+	+TM	TM/+	TM/TM	<i>P</i> -value
ToughA_loin ^a (kgF)	0.109	0.068	0.075	0.047	0.107
ToughB_loin ^a (kgF)	0.057	0.048	0.109	0.090	0.128
ToughA_leg (kgF) ^b	0.359 ^a	0.195 ^{ab}	0.240 ^a	0.123 ^b	0.020
IMF (%)	0.165	0.217	0.210	0.108	0.212
CT_MD (HU) ^c	4.61	6.54	5.50	3.85	0.58

^a Expressed in log values for LS means.

^b Means within row sharing a common superscript are not significantly different ($P>0.05$).

^c Hounsfield units (HU = (D − 1.0062)/0.00106, with density [D] in g/cm³).

Table 5

Genotype comparisons of predicted means for all sensory and preference attributes.

Attribute ^a	+/+	TM/TM	s.e.d.	<i>P</i> value—order	<i>P</i> value—genotype
Appearance	6.18	6.43	0.278	0.035	0.385
Flavour	6.18	6.84	0.388	0.213	0.103
Texture	5.93	6.27	0.498	0.138	0.496
Succulence	6.02	6.53	0.403	0.015	0.210

^a Scored on a subjective scale of 1 = very poor to 10 = excellent.

Table 6
Estimated variance components for all sensory and preference attributes.

Attribute	Scorer	Samples within genotype		Residual within genotype	
		+/+	TM/TM	+/+	TM/TM
Appearance	0.784	0.016	0.016	1.321	1.321
Flavour	0.000	0.507	0.507	1.485	1.485
Texture	0.176	1.123	1.123	1.535	1.535
Succulence	0.389	0.000	1.461	1.760	0.538

(Frazer, 1997; Jopson et al., 2001). In the current study, least-squares means for each genotype for both ToughA and ToughB were well below these levels, which may suggest no problems with toughness in any of the genotype groups. However, considering the raw data, 9 samples (of those with known genotypes) had ToughA values greater than 5.5 kgF, whilst 11 samples had ToughB measurements over 5.5 kgF and 3 had values over 8 kgF. Since it is important to maintain uniformity in eating quality, any genetic effects that result in increased variation in toughness may be undesirable. The 9 samples with higher ToughA included equal numbers ($n=4$) from the TM/+ and +/+ groups and one TM/TM sample, suggesting no link to genotype. However, of the 11 samples with higher ToughB values, 7 were from the TM/+ group (with one +/+ and three TM/TM samples also measuring >5.5 kgF). Of these, the three samples over 8 kgF were from two TM/+ lambs and one TM/TM lamb. However, it is important to consider that any extremes in meat quality may be influenced by the genetic background of the animals studied, rather than only this single QTL. In fact, comparing residual variances, none of the TM-QTL carrier groups displayed significantly greater variation in any of the meat quality traits than the non-carrier group, suggesting no detrimental effects of TM-QTL on uniformity of quality.

4.2. TM-QTL effects on subjective tests of sensory attributes

In order to put laboratory meat quality test results into perspective, it was necessary to conduct taste panel assessments despite the limited lamb numbers in genotype groups. Although the final numbers used from the two homozygous genotype groups may not be sufficient for a full statistical appraisal of TM-QTL effects on meat eating quality, it was expected that these results would give an indication of any obvious effects that may require further investigation. In retrospect, with the limited number of samples tested in the taste panel, it would have been more informative to choose lambs from the TM/+ and +/+ groups for comparison, since these are the genotypes that have shown the greatest differences in loin muscling (Macfarlane et al., 2010) and previous research on CLPG had implicated the genetic region as exhibiting paternal polar overdominance (Freking et al., 1998). However, before the mode of inheritance of TM-QTL was identified, it seemed a reasonable assumption that the two homozygote groups were likely to show the most divergent results, given that TM-QTL was known to be a separate mutation from CLPG.

Table 7
Pair-wise correlations between each objective and subjective measurement (those in bold are significantly different from zero).

	ToughA_loin	ToughB_loin	ToughA_leg	IMF	CT_MD	Appearance	Flavour	Texture
ToughB_loin	0.59							
ToughA_leg	0.22	0.20						
IMF	-0.32	-0.39	0.04					
CT_MD	-0.08	0.19	-0.22	-0.43				
Appearance ^a	-0.19	-	0.17	0.42	-0.27			
Flavour ^a	-0.16	-	0.23	0.51	-0.33	0.55		
Texture ^a	-0.36	-	0.23	0.26	-0.30	0.39	0.59	
Succulence ^a	-0.22	-	0.10	0.30	-0.16	0.43	0.60	0.82

^a Average value for each lamb across assessors. Also includes data from the 9 lambs with unknown genotype.

The design of sensory profiling experiments is challenging when wishing to compare a large number of hot samples and the design adopted in the current study was suboptimal. Although it would be unrealistic to expect an assessor to score forty samples at a single sitting, ideally all assessors should score every sample at some point, over a series of sessions. If it is not possible for all assessors to score every sample, then it is highly desirable to allocate samples to assessors in as optimal way as possible to ensure that: (a) each sample is scored by approximately the same number of assessors; and (b) comparisons of samples are not completely confounded with assessors and are as statistically efficient as possible. Maximising statistical efficiency requires each pair of samples to be assessed by the same assessor approximately the same number of times. The untrained nature of the taste panel may be resulting in less repeatable scores compared to a trained taste panel, making it more difficult to pick up significant differences between means. Correlations between sensory traits may also be inflated due to the fact that the different traits were scored at the same time and not independently.

While the current study was sufficiently sensitive to pick up differences between presentation orderings for some attributes, trends were not always consistent and spurious results could be attributed to the taste panel being untrained, at least for assessing lamb. There was no statistical evidence of differences between genotypes in mean levels of sensory attributes or preference. However, the available results suggest that there was a tendency for samples from TM/TM lambs to score more highly than those from +/+ lambs and there is no indication that TM-QTL may be having a negative effect on sensory attributes (when two copies of the QTL are carried). This result (for sensory texture score in particular) casts increasing doubt over the ToughB results, which suggested that TM/TM lambs had tougher loin muscle than +/+ lambs and did not agree with ToughA results. Such differences are also not apparent from the taste panel results.

There was some evidence for differences in variance components between genotypes for succulence, implying that TM-QTL carriers may be more variable than non-carriers for this trait, which would be undesirable. However, no differences in means or variances of IMF between genotypes were identified.

Ideally, a larger scale, more carefully designed, taste panel analysis should be conducted, using samples from lambs of all four genotypic classes, to identify any effects of mode of inheritance on means and variation of sensory attributes. It would also be interesting to combine this with further investigations of physical and chemical characteristics of the meat to gain more insight into the physiology underlying the few differences that were identified between genotypes and measurement methods. Several taste panel analyses have been conducted in previous studies in the literature to identify effects of the CLPG gene on meat eating quality (e.g. Duckett, Klein, Leckie, et al., 1998; Goodson, Miller, & Savell, 2001), however, these have only compared lambs with and without the CLPG phenotype, rather than all possible allele combinations. Little is known about how other muscling QTL/genes affect sensory meat eating quality characteristics.

4.3. Considerations for commercial use of TM-QTL

The results from this and previous studies (Lambe, Haresign, et al., 2010; Lambe, Macfarlane, et al., 2010; Macfarlane et al., 2009; Macfarlane et al., 2010) suggest that incorporation of TM-QTL into UK Texel sires would result in lambs (purebred and crossbred) with increased loin muscling, but would have little effect on meat quality, provided that meat was conditioned for more than seven days following high voltage electrical stimulation. A blueprint issued by the Meat and Livestock Commission in 1994 for producing high quality British lamb meat (Meat & Livestock Commission, 1994) recommended a minimum of 7 days maturation for carcasses that had been treated with high voltage electrical stimulation, with some additional benefit from a further 3 days maturation. However, in reality in the UK, the minimum time period from slaughter to eating is about three to four days (Vipond et al., 2004), with an average of around 5 days from slaughter to sale. These results provide further justification for enhanced chilling regimes.

The benefits accrued from the TM-QTL in terms of increased loin muscling, as well as the potential requirement for added costs for enhanced processing protocols, will depend not only on future carcass processing and payment systems, but also on the background frequency of TM-QTL in the current UK Texel population. This is not known and would not be easy to assess while a commercial genotyping test is unavailable. Texel sires are mated to around 23% of the total UK ewe flock (Pollott & Stone, 2006), so if the current QTL frequency is low, introducing the TM-QTL into Texel sires that are producing slaughter lambs could have a larger impact on loin muscle output across the UK industry. If current TM-QTL frequency is already high, however, this may mean that many TM-QTL carrier slaughter lambs are currently being processed in a manner that may be detrimental to meat quality (conditioning for <7 days). Further studies to fine-map the mutation, using SNP chips with high and lower density focussing on this chromosomal region, have the potential to produce a commercially-applicable genotyping test. This could then help to categorise Texel sires, to design breeding, management and processing strategies to best take advantage of the TM-QTL.

5. Conclusion

These results suggest that breeding programmes designed to produce Texel lambs carrying a single copy of TM-QTL, inherited from their sire, will increase loin muscling without any evidence of associated detrimental effects on meat tenderness (when meat is aged for at least 7 days) and intramuscular fat content. Analyses of the indirect effects of TM-QTL on ewe and lamb health and welfare traits are also underway. Results from these studies will be combined to assess whether this QTL should be recommended for use in the UK sheep industry and, if so, to determine the best design for breeding programmes that incorporate this QTL.

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