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The relationship between shear force, compression, collagen characteristics, desmin degradation and sarcomere length in lamb *biceps femoris*

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ABSTRACT

This study aimed to identity the relationships between known variants of tenderness (collagen content (total and soluble), desmin degradation and sarcomere length) and shear force and compression in the *biceps femoris* aged for 14 days from 112 mixed sex lambs. Desmin degradation was related to compression ($P < 0.05$) such that as desmin degradation increased compression decreased. Sarcomere length (SL) was related to shear force ($P < 0.05$), such that as SL increased shear force declined. Shear force was also related to compression ($P < 0.05$), and soluble collagen ($P < 0.05$), with male lambs producing higher shear force values than females (4.4 ± 1.72 N: $P < 0.05$) when adjusted for compression, sarcomere length and soluble collagen. The findings from this experiment indicate that the known variants (soluble collagen, sarcomere length and desmin degradation) are related to shear force and compression in *ovine biceps femoris*.

1. Introduction

Considerable research has been conducted to explain the variation in tenderness in different animal species: beef (Rhee, Wheeler, Shackelford, & Koohmaraie, 2004), pork (Wheeler, Shackelford, & Koohmaraie, 2000) and sheep meat (Starkey, Geesink, Collins, Oddy, & Hopkins, 2016; Hopkins, Allingham, Colgrave, & van de Ven, 2013) to name a few studies. All aforementioned authors have conducted research on multiple muscles to identify the variation in tenderness in the different animal species. As identified by Starkey, Geesink, Oddy, and Hopkins (2015), there are numerous factors which account for the variation in *ovine longissimus* tenderness, including desmin degradation, sarcomere length, collagen content and particle size, which is an indicator of the degree of myofibrillar degradation.

Desmin, which is a substrate for calpains, is important to cell integrity and muscle function (Geesink, Kuchay, Chishti, & Koohmaraie, 2006; Huff-Lonergan & Lonergan, 2005) and was significant in explaining the variation in tenderness in studies by Rhee et al. (2004), Starkey et al. (2015), Starkey et al. (2016) and Wheeler et al. (2000). The effect of sarcomere length on tenderness has been extensively researched (Hopkins & Thompson, 2001; Rhee et al., 2004; Smulders, Marsh, Swartz, Russell, & Hoenecke, 1990; Wheeler & Koohmaraie, 1994) and in some cases is curvilinear (Herring, Cassens, Suess, Brungardt, & Briskey, 1967). Rhee et al. (2004) found that sarcomere length was correlated to shear force results of the *longissimus* in beef, whereas Starkey et al. (2015) only found sarcomere length to be significant after 14 days of ageing in lamb *longissimus*. Collagen content (connective tissue) and solubility is responsible for the background toughness of meat (Bailey, 1972; Veiseth, Shackelford, Wheeler, & Koohmaraie, 2004). Warner et al. (2010) suggested for the *longissimus* muscle in lamb that total collagen content is of limited value when predicting tenderness, whereas the solubility of collagen would be expected to affect tenderness. There has been minimal study of the relationships for other ovine muscles such as the *biceps femoris* (e.g. Starkey et al., 2016) and none which have included measures of compression which can be indicative of collagen content depending on how this is measured (e.g. Hopkins et al., 2013).

The aim of this study was to identity the relationships between known variants of tenderness (collagen content (total and soluble),

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desmin degradation and sarcomere length) and how they affect shear force and compression in the *biceps femoris* of lamb, building on the study of Starkey et al. (2016).

2. Materials and methods

2.1. Sample collection

The animals that were used within this study ($n = 112$) were a subset of those used by Starkey et al. (2016), and comprised a mixture of sex (wethers and ewes) and age (7, 8, 9 and 11 months). Compression data were not available on the hoggets reported by Starkey et al. (2016), so they are not included in this paper.

All carcasses were trimmed according to AUS-MEAT specifications (Anonymous, 2005). Hot carcass weight (HCW) was recorded and the depth of tissue at the GR site (the depth of muscle and fat tissue from the surface of the carcass to the lateral surface of the twelfth rib (110-mm from the midline)) was measured using a GR knife. Briefly, both *biceps femoris* muscles were collected from each carcase at approximately 24 h after slaughter from 4 different kill groups at four different times (April to August). The left *biceps femoris* was trimmed and prepared into a 65 g sample for compression testing. The right *biceps femoris* was prepared as a 65 g shear force block with the trim being used for collagen content determination (20 g), sarcomere length and desmin degradation samples (5 g combined). All samples were aged for 14 days at 2 °C before being frozen at −22 °C.

2.2. Shear force determination

The method used to determine shear force was the same as described by Starkey et al. (2015). The samples (1 cm^2 cross section) were cooked in vacuum bags in a 70 °C water bath for 30 min before being stored over night at between 3 and 4 °C and subsequent determination of shear force. All shear force blocks were approximately 65 mm long, 43 mm wide and 23 mm high. The measurements were conducted on a Lloyd LRX Materials Testing Machine, fitted with a 500N load cell (Lloyd Instruments Ltd., Hampshire UK). The testing machine was fitted with a straight edged blade, which moved upwards at 100 mm/min and 6 technical replicates per sample were tested.

2.3. Compression determination

The compression determination method was similar to the methods used by Hope et al. (2013). The samples were cooked and chilled in the same way as the shear force samples. The same Lloyd LRX Materials Testing Machine was used, but it was fitted with a cylindrical rod (6.3 mm diameter) with a flat base and positioned approximately 10 mm from the base plate. The samples of similar dimensions to the shear force blocks were cut diagonally to form 2 wedge blocks parallel to the fibre direction. The thickness of the wedge was approximately 10 mm in height. The Lloyd was set to drive the rod at 50 mm/min and penetrate the wedge to a depth of 8 mm, then retract and re-penetrate the block in the same position at the same depth. The wedge was then repositioned and a new measurement was conducted. Three measurements were made per wedge and a total of 6 measurements were conducted per compression block. Compression was calculated as the product of hardness and cohesiveness, where hardness is the maximum height of the first force–deformation curve, and cohesiveness is the work done by the second compression stroke divided by the work done by the first compression stroke.

2.4. Measurement of collagen content, desmin degradation and sarcomere length

The method for determining total and soluble collagen was derived from the AOAC method 990.26 (AOAC, 2000), as previously described by Starkey et al. (2015). The desmin degradation method using SDS-PAGE and western blotting methods was as described by Geesink, Mareko, Morton, and Bickerstaffe (2001), with a detailed description provided by Starkey et al. (2015). Sarcomere length was determined using a similar method as described by Cross, West, and Dutson (1981) with full methodological details provided by Starkey et al. (2015).

2.5. Statistical analysis

Mixed models were fitted using ASReml-R (Butler, 2009) within the R software environment (R Core Team, 2014) to develop prediction models for shear force and compression traits.

Three different models were used to identify relationships with each model containing the covariates of sarcomere length, total collagen, soluble collagen, desmin degradation, hot GR (HGR), hot carcass weight (HCW) and lamb age. Random terms were included in all models, and they were *dam*, *sire* and *kill group*. The models are as follows;

Model 1. Compression = Sarcomere + Total Col + Sol Col + Desmin Degradation + Sex + HGR + HCW + Age + *dam* + *sire* + *kill group*

Model 2. Shear force = Sarcomere + Total Col + Sol Col + Desmin Degradation + Sex + HGR + HCW + Age + *dam* + *sire* + *kill group*

Model 3. Shear force = Compression + Sarcomere + Total Col + Sol Col + Desmin Degradation + Sex + HGR + HCW + Age + *dam* + *sire* + *kill group*

Each of the above models was simplified by sequentially removing non-marginal fixed effect terms not significant at the 0.05 level, using the Wald F statistic for small samples (Kenward & Roger, 1997). Marginal and conditional R^2 values, as defined by Nakagawa and Schielzeth (2013), were calculated for Models 1, 2 and 3 after removing non-significant terms. The marginal R^2 corresponds to the proportion of variance explained by the fixed effects alone whilst the conditional R^2 corresponds to the proportion of variance explained by the fixed and random effects jointly.

3. Results

As shown in Table 1, there was considerable variation in tenderness (shear force and compression) and related traits.

For model 1, sex and desmin degradation ($P < 0.05$) were significant. Females had higher compression values than males (Table 2) when adjusted for desmin degradation. The raw means for compression were 8.2 vs. 7.7 N with a s.e.d. of 0.17. The marginal $R^2 = 0.14$ and the conditional $R^2 = 0.18$. As desmin degradation increased the compression values decreased. There was an effect ($P < 0.05$) of dam, sire, and kill group. When individual traits were modelled (Model 2) against shear force, only sarcomere length was significant ($P < 0.05$) (Table 2), with a marginal $R^2 = 0.05$ and a conditional $R^2 = 0.40$. In this case there was an effect ($P < 0.05$) of dam, sire, and kill group. Compression ($P < 0.001$), sarcomere length ($P < 0.05$), soluble collagen ($P < 0.05$) and sex ($P < 0.05$) were all significant predictors of shear force (Model 3), with males on average having a higher shear force than females at common covariate values. The marginal $R^2 = 0.20$ and a conditional $R^2 = 0.49$. There was again an effect ($P < 0.05$) of dam, sire, and kill group.

Table 1
Unadjusted means, standard deviations and ranges for meat quality measures.

	Number	Mean ± SD	Range
SF (N)	112	39.6 ± 9.7	22.4–61.7
Compression (N)	112	7.9 ± 0.9	5.7–9.9
Total collagen (mg/g)	112	18.3 ± 5.5	9.5–40.6
Soluble collagen (mg/g)	112	5.3 ± 2.3	1.2–9.8
Desmin (Degraded/intact) (%)	112	3.5 ± 3.0	1.1–19.9
Sarcomere length (μm)	112	1.6 ± 0.1	1.34–2.1