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Do sarcomere length, collagen content, pH, intramuscular fat and desmin degradation explain variation in the tenderness of three *ovine* muscles?

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ABSTRACT

The *longissimus* (n = 118) (LL), *semimembranosus* (n = 104) (SM) and *biceps femoris* (n = 134) (BF) muscles were collected from lamb and sheep carcases and aged for 5 days (LL and SM) and 14 days (BF) to study the impact of muscle characteristics on tenderness as assessed by shear force (SF) and sensory evaluation. The impact of gender, animal age, collagen content, sarcomere length (SL), desmin degradation, ultimate pH and intramuscular fat (IMF) on tenderness was examined. The main factors which influenced SF of the LL were IMF, SL and desmin degradation, but for sensory tenderness, IMF, ultimate pH and gender were the main factors. The SF and sensory tenderness of the SM was best predicted by the degree of desmin degradation. For the BF soluble collagen and animal age both influenced SF. Different factors affect tenderness across muscles and not one prediction model applied across all muscles equally well.

1. Introduction

The eating quality of meat is determined by the tenderness, juiciness and flavour (Thompson, 2002). Of these traits, tenderness is affected by both production and processing factors (Young, Hopkins, & Pethick, 2005). Since there is variation in the tenderness of lamb at the retail level (Safari, Channon, Hopkins, Hall, & van de Ven, 2002), it is important to understand what interactions occur within and across different muscles, and how these interactions influence the variation in tenderness. There are three major factors which can impact on the tenderness of meat; these include collagen content and solubility (background toughness), muscle shortening (toughening) and ageing (tenderisation) (Hopkins & Geesink, 2009). The latter two factors (muscle shortening and ageing) take place during *post-mortem* storage (Hopkins & Thompson, 2001). The impact of sarcomere length has been researched extensively (Hopkins & Thompson, 2001; Rhee, Wheeler, Shackelford, & Koohmaraie, 2004; Smulders, Marsh, Swartz, Russell, & Hoenecke, 1990; Wheeler & Koohmaraie, 1994). As such Rhee et al. (2004) found that overall sarcomere length was significantly correlated to tenderness.

The impact of ageing (proteolysis) on tenderness is thought to be due to weakening of the myofibrillar structure as result of degradation of muscle proteins, like titin, nebulin and desmin (Hopkins & Thompson, 2002). A number of methodologies have been used to study proteolysis including the study of specific proteins (Hopkins & Thompson, 2002). One such protein of interest is desmin, because it is a calpain substrate (Geesink, Kuchay, Chishti, & Koohmaraie, 2006; Huff-Lonergan & Lonergan, 2005), and because it is important to the function and integrity of muscle cells. In a study conducted by Starkey, Geesink, Oddy, and Hopkins (2015) desmin degradation was the most important factor for explaining variation in shear force over different ageing periods in lamb *longissimus*.

Connective tissue (i.e. collagen) content and solubility impact on tenderness and this is referred to as background toughness (Veiseth, Shackelford, Wheeler, & Koohmaraie, 2004). The solubility of collagen is affected by a number of factors such as animal age (Young & Braggins, 1993), sex and muscle type (Wheeler, Shackelford, & Koohmaraie, 2000), and this leads to a variation in tenderness. The extent of this effect varies however, according to the muscle under study. For the *longissimus* Warner et al. (2010) suggested that total collagen content is of limited value when predicting tenderness, whereas the level of soluble collagen would be expected to affect tenderness.

For lamb (*ovine*) there are no published studies which have examined the effect of indices of muscle structure and degradation across a number of muscles. The study of Starkey et al. (2015) only focussed on the tenderness of the *longissimus* muscle. For other species such as pigs, Wheeler et al. (2000) reported that sarcomere length, total collagen and proteolysis (quantified as desmin degradation) when combined could explain more than 50% of the variation in sensory tenderness across 5 muscles.

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This was for muscle aged for 1 day and the relationship varied according to muscle.

Thus, the objective of the experiment detailed in this paper was to examine the effect of sarcomere length, collagen content, pH, intramuscular fat and indicators of proteolysis on the variation in lamb meat tenderness (objectively and subjectively measured) for three different lamb muscles: *longissimus thoracis et lumborum* (loin), *semimembranosus* (topside) and *biceps femoris* (silverside).

2. Materials and methods

2.1. Animal background and slaughter

Two hundred and thirty one mixed sex lambs and 28 male hoggets (first permanent incisor) from the Sheep CRC Information Nucleus flock (van der Werf, Kinghorn, & Banks, 2010) were sampled at slaughter in 2011. The animals were bred at the UNE Kirby farm (Armidale NSW Australia) and slaughtered from April to August in 4 separate kill groups at a processing plant in Tamworth, NSW. The lambs were offspring of 10 different sire breeds which included Merino, maternal and terminal sire types. The hoggets (castrated males) were the progeny of 16 different sires, all Merino, joined to Merino dams, and were all slaughtered as part of the third lamb slaughter. The slaughter process consisted of electrical stunning, followed by exsanguination aided by a pre-dressing medium voltage electrical stimulation system. Carcases were trimmed to AUS-MEAT specifications (Anonymous, 2005) and weighed to determine hot carcase weights (HCW), and chilled for 24 h at 2 °C.

The initial pH and temperature recordings were taken as soon as the first of the carcases entered the chiller. This was conducted on the left hand side of the m. *longissimus lumborum* at the caudal end over the lumbar sacral junction, as described by Hopkins et al. (2011). Three subsequent measures were recorded over the next 3 h with approximately 50 min between measurements. These measurements were used to calculate the Temperature@pH 6 and pH@Temperature18 values as per van de Ven, Pearce, and Hopkins (2014). The ultimate pH was measured approximately 24 h post-mortem, on the same section of muscle. Muscle pH was recorded using a glass combination pH probe (potassium chloride) (Ionode intermediate junction pH electrode TPS, Pty Ltd., Brisbane) attached to a data recording metre (TPS WP-40). The temperature data was recorded using a steel probe attached to the same metre. The pH metre was calibrated before each set of recordings by using buffers at pH 4 and 6.88 at room temperature (approximately 20 °C) and at 24 h at chiller temperature (approximately 5 °C).

2.2. Sampling

The hind quarter (leg and aitch bone) was separated from the saddle and rack and the forequarter (shoulder), as described by Pannier, Gardner et al. (2014). The left and right topsides were removed from the hind legs. Then the *gracillus* and *adductor* muscles were removed to leave the *semimembranosus* (SM) which was vacuum packed. The *semimembranosus* from the left legs were used for subsequent sensory testing, these being a subset of those reported on by Pannier, Gardner et al. (2014). The *semimembranosus* from the right leg was used for subsequent shear force testing. Both samples were aged for 5 days at 2 °C then frozen at −22 °C. Samples were taken after 5 days of ageing for determination of collagen content (20 g), sarcomere length measurement and measurement of desmin degradation (5 g combined) then frozen at −22 °C. The *biceps femoris* muscle was removed from the right side and prepared into a 65 g shear force block which was aged for 14 days at 2 °C. Samples were also collected for determination of collagen content (20 g), sarcomere length measurement and measurement of desmin degradation (5 g combined) after 14 days of ageing and then frozen at −22 °C.

The right side *longissimus lumborum* was boned out from the 12/13th rib to the lumbar sacral junction (Pannier, Gardner et al., 2014). The subcutaneous fat and epimysium were removed from the *longissimus*, which was then divided into 3 sections. The caudal end was used for shear force testing (65 g block) after 5 days of ageing at 2 °C. The cranial end of the *longissimus* was used for intramuscular fat (IMF) (20 g) analysis, collagen content determination (20 g), sarcomere length measurement and measurement of desmin degradation (combined 5 g). A portion for sensory testing was prepared from the left side *longissimus* from the 5th rib to the lumbar sacral junction and was a subset from the study conducted by Pannier, Gardner et al. (2014). After the muscle samples were aged for the allotted 5 days, they were frozen and stored at −22 °C until testing.

The same 3 muscles (*semimembranosus*, *longissimus* and *biceps femoris*) were removed from the hogget carcases as described. The samples collected from the carcases were the same as from the lamb samples, with; *longissimus* (shear force, sensory tenderness, sarcomere length, IMF, collagen and desmin), *semimembranosus* (shear force, sensory tenderness, sarcomere length, collagen and desmin) and *biceps femoris* (shear force, sarcomere length, collagen and desmin) samples collected. After determination of the shear force, one hundred and eighteen samples (103 lamb, 15 hogget) from the *longissimus*, 104 (90 lamb, 14 hogget) from the *semimembranosus* and 134 samples (119 lamb, 15 hogget) from the *biceps femoris* were selected for detailed analysis. Samples were selected to allow for an even distribution from all three muscles from the four slaughters across the range in shear force to ensure there was sufficient variation to develop models.

2.3. Shear force determination

There were two different methods used by 3 different laboratories to assess shear force. The first method used for the determination of shear force (SF) was described by Starkey et al. (2015). In this method samples were cooked in a 70 °C water bath for a period of 30 min and stored overnight at 3–4 °C before shear force determinations. This was used for the *biceps femoris* samples only. The second method was described by Hopkins, Toohey, Warner, Kerr, and van de Ven (2010) and in this method *longissimus* and *semimembranosus* samples were cooked in a 71 °C water bath for 35 min and stored overnight at 3–4 °C. The blocks were approximately 65 mm long, 43 mm wide and 23 mm high and weighed approximately 65 g. All shear force measurements were conducted on a Lloyd Instruments LRX Materials Testing Machine fitted with a 500 N load cell (Lloyd Instruments Ltd., Hampshire UK). The machine setups were slightly different, with the laboratory which tested the *biceps femoris* using the method described by Starkey et al. (2015) where a straight edged blade moved upward at 100 mm/min. The other two laboratories used the method described by Hopkins et al. (2010) for measurement of the *longissimus* and *semimembranosus* samples. All laboratories used the following procedure with; six subsamples were tested with a rectangular cross section of 1 cm^2. The fibre direction ran parallel to the length of the sample and at right angles to the shearing surface. The amount of force required to cut through the fibres was measured as peak force in Newtons. The average of 6 subsamples was recorded.

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

The method for the determination of sarcomere length (SL) was similar to that previously described by Cross, West, and Dutson (1981), with the details given by Starkey et al. (2015). The method to determine total and soluble collagen was derived from AOAC method 990.26 (AOAC, 2000) as previously described by Starkey et al. (2015). Desmin degradation was determined by using SDS-PAGE and Western blotting methods described by Geesink, Mareko, Morton, and Bickerstaffe (2001) with full detail provided by Starkey et al. (2015).