EXPLAINING THE VARIATION IN SHEAR FORCE OF LAMB *LONGISSIMUS* MUSCLE USING INDICATORS OF PROTEIN DEGRADATION, MUSCLE CONTRACTION AND CONNECTIVE TISSUE CHARACTERISTICS

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Abstract – Meat tenderness is known to be affected by sarcomere length (SL), proteolysis and collagen content (CC). 60 lambs were slaughtered and the *Longissimus* muscle was sampled. Shear force (SF), SL, proteolysis (desmin, PSA) and CC samples were taken after the allotted aging periods (1, 7, and 14 days). Proteolysis explained a large part of the variation in tenderness (approximately 35%). Other factors (CC, SL) combined with proteolysis (PSA, desmin) explained just under 40% of the variation of meat tenderness. The main conclusion that can be drawn from this study is that ageing response i.e. post-mortem proteolysis is the dominant factor determining lamb loin tenderness.

Key Words – Collagen, Lamb, Tenderness, Proteolysis, Sarcomere length

- INTRODUCTION

Meat tenderness (MT) has been established as one of the most important traits of meat quality [1,2]. Variation in meat tenderness can have a negative impact on both the industry and consumers. There are three main factors which impact on the tenderness of meat. These are: the background toughness (collagen content), muscle contraction during the onset of rigor (sarcomere length) and the improvement in tenderness during ageing post-mortem (p.m.) [3].

During ageing, structural muscle proteins are degraded [4]. Several authors suggest that this is one of the key factors responsible for the variation in meat tenderness [5]. Desmin is a key protein for the maintenance of structural integrity of the

muscle and is a substrate of the calpain enzymes [6,7].

A common method to assess the impact of p.m. proteolysis is to measure the fragility of the myofibrillar structure using techniques like the Myofibrillar Fragmentation Index (MFI), or Particle Size Analysis (PSA) [8].

Rhee *et al* [9] conducted research on beef *longissimus* muscle, but only aged the samples for one time period (14 days). In their study, post-mortem proteolysis (desmin degradation) explained about 36% of the variation in shear force, and only a minor part of the variation could be attributed to variation in sarcomere length or collagen content.

The objective of this study was to determine the importance of different factors that may affect the tenderness of lamb *longissimus* muscle after different ageing periods. To this end, lamb

longissimus muscle was aged for 1, 7 and 14 days and sampled for determination of shear force, indicators of *post-mortem* proteolysis, collagen content and sarcomere length.

- MATERIALS AND METHODS

The lambs that were used in this study were sourced from the Trangie Sheep CRC INF flock [10]. Mixed sex lambs (n = 60) were transported from Trangie to Dubbo and slaughtered in December 2011. Lambs were slaughtered at a target carcase weight of 21-22kg. Carcases were processed under normal commercial abattoir procedures. The carcases were subjected to electrical stimulation, and chilled at approximately 5^{o}C.

pH and temperature declines were determined. The first recording was taken as soon as the carcases entered the chiller. Three subsequent measurements were made over the next 3 hours with approximately 50 minutes between measures. These measures of temperature and pH plus ultimate pH values were used to calculate the temperature of the muscle at rigor (Temp@pH6 [11]. Ultimate pH was measured at approximately 24 hours *post-mortem*. Muscle pH was measured using a glass combination pH probe (potassium chloride)(Ionode intermediate junction pH electrode TPS, Pty Ltd., Brisbane) attached to a data recording meter (TPS WP-80). The temperature data was recorded using a steel probe attached to the same meter. pH meters were calibrated regularly before each set of recordings by using buffers of pH 4 and 6.8 at room temperature and at 24 hours at chiller temperature.

Muscle samples were taken from the M. *longissimus*. The muscle samples were separated into 3 different sections (Cadual, Medial and Cranial). Each of the different sections was vacuum packed and randomly assigned to different ageing periods (1, 7 and 14 day at 3-4^{o}C). After the ageing period, subsamples were taken for the different analyses, and stored at -22^{o}C until use. Shear force (SF) was determined using samples weighing approximately 65 gram block and each block was approximately 65mm long, 45mm wide and 25mm high. These samples were cooked in a water bath at 70^{o}C for 30 min., patted dry and stored overnight at 3-4^{o}C. Shear force (N) determination was conducted as described by Perry *et al.* [12].

Sarcomere lengths were determined by the filar micrometer method described by Cross *et al*. [13]. The method for determination of total and soluble collagen was based on AOAC method 990.26 [14]. Total collagen was determined in triplicate on 0.10 g of freeze dried muscle powder by determining the hydroxyproline content after hydrolysis at 105°C with 3.5 M H_2SO_4 for 16 h. Total collagen content is expressed as mg/kg dry matter. For determination of soluble collagen, 1.5 g of freeze dried muscle powder was suspended in 10 mL distilled water and heated in a water bath at 80°C for 2 h with occasional mixing. After heating, the samples were filtered through a Whatman filter. The hydroxyproline content in the filtrate was determined in duplicate after hydrolysis at 105°C with 3.5 M H_2SO_4 for 16 h. Soluble collagen content is expressed as mg/kg dry matter.

Degradation of desmin was determined by using the SDS-PAGE and Western blotting as described by Geesink *et al*. [15] using a mouse monoclonal anti-desmin antibody (DE-U-10; dilution 1:2500; Sigma-Aldrich, St. Louis, MO) The secondary antibody used was alkaline phosphatase labeled goat anti-mouse IgG (A3562; dilution 1:10,000; Sigma-Aldrich, St. Louis, MO). Blots were developed using an alkaline phosphatase conjugate substrate kit (BioRad Laboratories, Hercules, CA). The intensity of the bands corresponding to intact and degraded desmin was quantified using Bio-1D software (Vilber Lourmat, Eberhardzell Germany). The amount of desmin degradation was expressed as the ratio of degraded desmin and intact desmin.

PSA was conducted using the method described by Karumendu *et al.* [16] with 1g of sample homogenized at 16,000 rpm. Stepwise linear regression and analysis of variance was conducted

using Minitab version 14. When statistically significant differences were detected ($P < 0.05$), comparisons were performed using a Pearson correlation.

- RESULTS AND DISCUSSION

The pH declines were, on average, not indicative of either cold- or heat-shortening conditions with a temperature at pH 6 of 22.4°C.The mean ultimate pH was 5.51 ranging from 5.45 - 5.72. As expected, ageing period had a significant effect on SF. Location within the muscle also affected SF, with the medial section being more tender than the cranial and the caudal segments of the loin. The changes in the different traits across the different ageing periods are given in Table 1.

Table 1 Tenderness (SF) and related traits in lamb longissimus muscle after 1, 7 and 14 days of ageing (means and standard deviations)

	Ageing period		
Factor	1	7	14
SF (N)	35.5 ± 8.27	24.8 ± 5.31	23.8 ± 4.72
PSA (µm)	152.1 ± 38.4	83.9 ± 24.9	75.7 ± 21.9
Sarc (µm)	1.77 ± 0.15	1.75 ± 0.12	1.80 ± 0.14
Desmin (deg/intact)	0.37 ± 0.31	1.67 ± 1.23	2.48 ± 2.05
Total CT (mg/kg)	12.8 ± 2.2	12.9 ± 2.0	14.1 ± 2.0
Sol CT (mg/kg)	2.00 ± 0.57	2.60 ± 0.64	2.56 ± 0.49

PSA values declined over the different aging periods confirming the results of Karumendu *et al.* [16] for lamb longissimus muscle and Lametsch *et al.* [8] for porcine *longissimus*. The biggest decrease in PSA values occurred between 1 and 7 days p.m., with relatively little decrease between 7 and 14 days p.m. In contrast, the desmin degradation values increased to almost the same extent between 1 and 7 days p.m. and 7 and 14 days p.m. However, overall the desmin degradation values ranged from 0.02 to 10.1. For this reason, desmin values were log transformed to linearise the data allowing for a more uniform fit in the model. Pearson correlation coefficients and their significance were calculated (Table 2). Subsequently, stepwise linear regression revealed that 42% of the variation in SF could be explained by the factors listed in Table 2.

Table 2 Pearson correlations (r) between factors determining meat tenderness

	SF	PSA	Log Desmin	Sarc	Temp@pH6
PSA	0.587				
	P=0.000				
Log Desmin	0.520	0.623			
	P=0.000	P=0.000			
Sarc	-0.138	-0.023	0.024		
	P=0.065	P=0.764	P=0.753		
Temp@pH6	-0.135	-0.099	0	-0.079	
	P=0.071	P=0.194	P=0.995	P=0.291	
Sol CT	-0.287	-0.303	0.246	0.045	-0.068
	P=0.000	P=0.000	P=0.001	P=0.552	P=0.367

PSA and Log Desmin were both highly significantly correlated to SF and each other, suggesting that they are interchangeable as measures of post-mortem proteolysis.
Nevertheless, when combined, PSA and Log desmin explained a larger part of the variation in

SF, than each of these factors separately (Table 2).
The stepwise linear model that explained the largest part of the variation in SF (42 %) was as follows:

SF = 54.3+0.0175*PSA-3.64*LogDesmin-8.06* Sarc-0.731*Temp@pH6-1.46*Sol CT

The relation between the actual SF values and the values calculated using this model is given in Table 3 and Figure 1.

Table 3 Actual and calculated shear force over the different ageing periods (means and standard deviation)

	Ageing period		
Factor	1	7	14
SF	35.5 ± 8.27	24.8 ± 5.31	23.8 ± 4.72
Calculated SF	32.4 ± 5.26	25.5 ± 2.86	24.7 ± 2.56

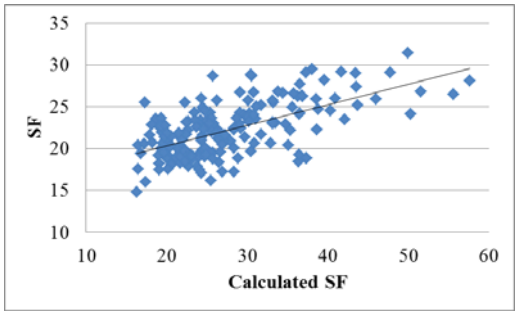

Figure 1. Correlation between actual and calculated shear force.

- CONCLUSION

The main conclusion to be drawn from the study is that the ageing response (proteolysis) is the dominant factor determining the tenderness of lamb loins. Therefore, methods to improve the tenderness of lamb loins should focus on the variation in the ageing response.

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