

INTERRELATIONSHIP BETWEEN MEASURES OF COLLAGEN, COMPRESSION, SHEAR FORCE AND TENDERNESS

David L. Hopkins^{1,2}, Peter G. Allingham³, Michelle Colgrave³ and Remy J. van de Ven⁴

¹ Australian CRC for Sheep Industry Innovation, Homestead Building, UNE, Armidale, NSW 2350, Australia

² NSW Department of Primary Industries, Centre for Red Meat and Sheep Development, Cowra, NSW 2794, Australia

³ CSIRO Animal, Food and Health Sciences, Queensland Bioscience Precinct, 306 Carmody Rd, St Lucia, Qld 4067, Australia

⁴ NSW Department of Primary Industries, Orange Agricultural Institute, Forest Road, Orange NSW 2800, Australia

Abstract – This study examined the relationship between collagen content as determined by hydroxyproline assay with other measures of connective tissue, shear force and tenderness in lamb muscle. Samples were taken from both m *longissimus lumborum* (LL, loin) and the m. *semimembranosus* (SM, topside) of 99 lambs. Sensory tenderness and compression of the LL were not correlated to any measure of collagen or connective tissue, with one exception where compression was correlated ($r = -0.28$; $P < 0.05$) to the percentage of connective tissue determined by imaging. There was no correlation between SM shear force or collagen concentration. The data suggest that measurement of collagen concentration did not explain the variation in shear force and sensory tenderness observed in the meat from lambs.

Key Words – Connective tissue, Lamb, Muscle.

• INTRODUCTION

Recent studies suggest that a detrimental impact on eating quality of lamb loins can occur when sheep sires with increased muscling are used and this has been partially explained by differences in connective tissue morphology [1] and a reduction in fatness [2] in the loin. Further to this, the *semimembranosus* muscle upon which the topside cut is based has been shown to be tougher in the progeny of Poll Dorset rams selected for muscling [3]. Intramuscular connective tissue (IMCT) is responsible for the background toughness of cooked meat and it has been proposed that subtle variations in this component of meat may arise due to factors such as levels of pre-slaughter nutrition [4]. Additionally during post-mortem ageing of meat there have been some reports of degradation of IMCT [5], but any contribution to improvement in cooked meat tenderness is equivocal. Collagen is the major component of the IMCT, being 2-7% of the fat free dry weight of muscles such as the loin (*longissimus* muscle, LL) [6]. Young & Braggins [7] showed that the collagen content of the topside was correlated to tenderness as assessed by a taste panel, and that tenderness measured by shear force was correlated with collagen solubility. However, measurement of collagen solubility is expensive and time consuming and so after developing a sensitive assay for the determination of collagen content [8] a study was undertaken to examine how the results of the assay related to other measures of connective tissue, shear force and tenderness. To provide variation in potential collagen characteristics lambs representing a wide genetic base and exposed to differing growth paths were used. This was a precursor for potential use of the assay in a program to phenotype 2,000 lambs per year for five years as part of the Australian Sheep CRC Information Nucleus [9].

- MATERIALS AND METHODS

Experimental details on the lambs have been published previously [10]. A sub-set of animals ($n = 99$) which were the progeny of 20 Poll Dorset sires and were weaned at 30 kg live weight were used. Within the group, a subset of lambs was either maintained at weaning weight for 55 days then re-alimented or was fully fed until all lambs reached a 45 kg target weight for slaughter. Assignment to sire type group was based on the sire Australian Sheep Breeding Values (ASBV's) and consisted of four groups of five sires selected for 1) high PWWT (growth), 2) high PEMD (muscling), 3) high PWWT and PEMD and 4) low PWWT and average PEMD as controls.

The sampling of lamb carcasses for meat quality measurements has been reported previously [2]. A 65 g block of the *m longissimus lumborum* (LL) was removed at 24 post-mortem from the left short loin and frozen (-20°C). From the left side hindleg the *m. semimembranosus* (SM) was removed vacuum packed and held chilled ($4-5^{\circ}\text{C}$) until freezing on day 5 post-mortem. Samples of LL were tested for compression and the SM for shear force. Short loins from the right side were used for consumer testing as outlined by Thompson *et al.* [11]. Intramuscular fat (IMF) content was determined on SM and LL samples on a whole tissue basis (IMFT). The concentration of hydroxyproline (nmol/L) was determined as previously described [8]. At 24 h post mortem, samples of LL were prepared for histology [1]. Each section was stained and imaged (Olympus microscope using a 4x lens and a Qimaging Micropublisher 3.3RTV camera) and the average perimysial seam thickness (ST) was calculated from the total of all seam thickness measures (TST) divided by the total number of perimysial segments measured. Likewise, the average fascicular width (FW) was calculated from the sum of all chords (total fascicular width, TFW), calculated from the circumference travelled between consecutive perimysial seams, divided by the number of chords measured. Integrating across all circles provided total pixels for each colour muscle component and connective tissue area versus total tissue area within the confines of the outermost circle. The connective tissue area proportion of the total muscle image as determined from this process was expressed as the percent connective tissue (PCT).

Seven dependent variables were jointly analysed using multi-variate linear mixed model (MLMM) analysis in ASReml. These variables were: compression of the LL in Newtons, collagen content mg/kg of the LL on a wet basis (CollLLWB), collagen content mg/kg of the LL on a dry de-fatted basis (CollLLFB), percentage of LL image that was connective tissue (PCT), average perimysial seam thickness (ST), average fascicular width (FW) scaled by dividing by 10 and tenderness (assessed by consumers). Each variate was allowed to vary separately with each level of; treatment group, sex (wether, ewe), age of animal, siretype and sire (fitting sire as a random effect), and varying linearly with the co-variates age of animal, birthweight, hot carcass weight, sire ASBV's PEMD, PFAT and PWWT, and IMFT. This model was subsequently reduced in complexity by removing non-significant terms. The same model was applied to three other dependent variables, collagen content mg/kg of the SM on a wet basis (CollSMWB), collagen content mg/kg of the SM on a dry de-fatted basis (CollSMFB) and shear force of the SM.

- RESULTS AND DISCUSSION

The lambs sampled for this work had a mean age of 219 days and ranged in age from 197-241 days. Hot carcass weight was 21.5 kg with a range of 16.4-28.2 kg. Treatment impacted significantly ($P < 0.05$) on CollLLWB, CollLLFB, PCT and scaled FW values, such that lambs grown on full feed had more collagen and more connective tissue as a proportion of muscle area, but with thinner fascicular bundle width. There was also a significant effect of

lamb age ($P < 0.05$) on CollLLFB such that the collagen content on a fat free basis increased as lamb age increased (0.46 ± 0.23 mg/kg collagen per 1 day of age). Of the variates after adjusting for fixed effects, CollLLWB and CollLLFB were shown to be highly correlated ($r = 0.99$). The correlation between PCT and ST was positive (0.42), whilst the correlations between PCT and scaled FW and between ST and scaled FW were negative (-0.44, and -0.57 respectively) all at the $P = 0.05$ level. Sensory tenderness and compression were not correlated to any measure of collagen or connective tissue with one exception where compression was correlated ($r = -0.28$; $P < 0.05$) to the percentage of connective tissue (PCT) determined by imaging. Intramuscular fat (IMFT) was linearly correlated ($P < 0.05$) to sensory tenderness and compression, such that a 1% increase in IMFT increased the tenderness score by 2.3 ± 0.83 units and reduced compression by 0.73 ± 0.21 Newtons. At the mean value of IMF of 3.5% the predicted mean tenderness score is 68.8 ± 0.86 and for compression 14.8 ± 0.22 N. As IMFT increased the concentration of collagen on a whole tissue basis (CollSMWB) decreased ($P < 0.05$). The traits CollSMWB and CollSMFB were highly correlated ($r = 0.99$), but there was no significant correlation between these traits ($r = 0.19$ and 0.22 respectively) and SM shear force.

This study was designed to investigate the interrelationships between measures of texture and connective tissue. Unfortunately these measures did not relate to those of collagen concentration at least when measured on loin muscle. The lower relative percentage of heat-stable collagen in the m. *longissimus* may have influenced this outcome, but there was also no relationship between collagen concentration and either sensory tenderness or compression. There was however a significant and negative relationship between connective tissue area as a proportion of the total muscle image (PCT) and compression which conforms to expectation. Unfortunately this study demonstrated that collagen concentration did not relate to traits like shear force or sensory tenderness and supports the conclusion of Monin, & Ouali [12] that qualitative characteristics of collagen (such as the nature of cross-links) are more likely to explain the variation in tenderness between carcasses.

• CONCLUSION

Based on the relationships found in this study it is concluded that measurement of collagen concentration would not provide meaningful explanation of variation in shear force or sensory tenderness in meat from lambs generated in the Information Nucleus. Coupled with initial genetic analysis this suggested that measures of connective tissue should not be continued.

ACKNOWLEDGEMENTS

The authors thank staff at the NSW Department of Primary Industries, Cowra and Mal Boyce, Murdoch University WA, for their expert technical support in the collection, drying and milling of muscle samples. This research was partially funded by the Sheep CRC.

REFERENCES

1. Allingham, P. G., Gardner, G. E., Taylor, M., Hegarty, R. S. & Harper, G. S. (2006). The effect of sire genotype and plane of nutrition on the fascicular structure of longissimus thoracis et lumborum muscle in the context of lamb meat quality. *Australian Journal of Agricultural Research* 57: 641-650.
2. Hopkins, D. L., Stanley, D. F., Toohey, E. S., Gardner, G. E., Pethick, D. W. & van de Ven, R. (2007). Sire and growth path effects on sheep meat production. 2. Meat and eating quality. *Australian Journal of Experimental Agriculture* 47: 1219-1228.
3. Hopkins, D. L., Stanley, D. F., Martin, L. C., Toohey, E. S. & Gilmour, A. R. (2007). Genotype and age effects on sheep meat production. 3. Meat quality. *Australian Journal of Experimental*

- Agriculture 47: 1155-1164.
4. Sylvestre, M. N., Balcerzak, D., Feidt, C., Baracos, V. E. & Bellut, J. B. (2002). Elevated rate of collagen solubilization and postmortem degradation in muscles of lambs with high growth rates: possible relationship with activity of matrix metalloproteinase's. *Journal of Animal Science* 80: 1871–1878.
 5. Nishimura, T., Liu, A., Hattori, A. & Takahashi, K. (1998). Changes in mechanical strength of intramuscular connective tissue during post-mortem aging of beef. *Journal of Animal Science* 76: 528-532.
 6. Purslow, P. P. (2002). The structure and functional significance of variations in the connective tissue within muscle. *Comparative Biochemistry and Physiology* 133: 947-966.
 7. Young, O. A. & Braggins, T. J. (1993). Tenderness of ovine semimembranosus: is collagen concentration or solubility the critical factor? *Meat Science* 35: 213–222.
 8. Colgrave, M. L., Allingham, P. G. & Jones, A. (2008). Hydroxyproline quantification for the estimation of collagen in tissue using multiple reaction mass spectrometry. *Journal of Chromatography A* 1212: 150-153.
 9. Pethick, D. W., Jacob, R. H., McDonagh, M. B., O'Halloran, W. J., Ball, A. J. & Hopkins, D. L. (2009). A new generation meat program in Australia within the Co-operative Research Centre for Sheep Industry Innovation. In *Proceedings of the 69th New Zealand Society of Animal Production* (pp 96-100) Christchurch, New Zealand.
 10. Hopkins, D. L., Stanley, D. F., Martin, L. C., Ponnampalam, E. N., & van de Ven, R. (2007). Sire and growth path effects on sheep meat production. 1. Growth and carcass characteristics. *Australian Journal of Experimental Agriculture* 47: 1208-1218.
 11. Thompson, J. M., Hopkins, D. L., D'Sousa, D., Walker, P. J. & Pethick, D. W. (2005). The impact of processing variables on the consumer perception of sheep meat eating quality. *Australian Journal of Experimental Agriculture* 45: 561–573.
 12. Monin, G. & Ouali, A. (1991). Muscle differentiation and meat quality. In 'Developments in Meat Science – 5' (Ed RA Lawrie) pp. 89-158. (Elsevier Applied Science: Essex, UK).