PE7.47 High temperature conditioning of the lamb carcass causes heat-toughening and high water loss 436.00

Robyn Warner (1) robyn.warner@dpi.vic.gov.au, M Kerr(1), ,
(1)Victorian Department of Primary Industries, Australia

***Abstract*—Fast pH fall and high muscle temperatures are known to cause quality problems in pig meat but this has not been reported in lamb meat. The aim was to determine the effect of post-slaughter holding temperature and muscle stretching on tenderness and water-holding capacity and the influence of protein denaturation in four different hind-leg muscles in the sheep. 48 lambs were slaughtered and the carcasses randomly allocated to treatments of +/- medium voltage electrical stimulation, a holding temperature of 2°C or 37°C for 5 hrs post-slaughter and +/- stretch applied to one side of the carcass. pH and temperature in the *longissimus* muscle was measured post-slaughter and samples removed at 1 and 8 days post-slaughter for quality and protein denaturation measurements. The stretch treatment resulted in longer sarcomeres in the *gluteus medius* (GM), *semimembranosus* (SM) and *semitendinosus* (ST) and resulted in shortening in the *rectus femoris* (RF). The 37°C holding temperature resulted in tougher meat in the unstretched GM and SM muscles after 0 and 8 days of ageing relative to the 2°C holding temperature but the stretched muscles held at 37°C were more tender after 0 and 8 days of ageing then the 2°C temperature (P<0.05). The muscles in carcasses held at 37°C for 5 hrs prior to chilling had higher purge, cooking loss and surface exudate then the muscles held at 2°C post-slaughter (P<0.05 for all). For the 37°C temperature SM and ST muscles, the non-stretched treatment produced higher purge and surface exudate then the stretch treatment (P<0.1 for all). In the SM, ST and GM muscles, the 37°C holding temperature resulted in lower sarcoplasmic protein solubility and lower myofibrillar ATPase activity (P<0.01 for all), particularly in the stretched muscles, indicating extensive protein denaturation. In conclusion, heat-toughening occurred in the non-stretched lamb muscle subjected to a post-slaughter holding temperature of 37°C. The high holding temperature also resulted in excessive water loss and protein denaturation.**

R. D. Warner is with the Victorian Department of Primary Industries, Werribee, 3030, Australia (Telephone: +61 3 97420477, Fax: +61 3 97420400, e-mail: Robyn.Warner@dpi.vic.gov.au).

M.A. Kerr. Kearney is with the Victorian Department of Primary Industries, Werribee, 3030, Australia (e-mail: matthew..kerr@dpi.vic.gov.au).

***Index Terms*— heat-toughening, protein denaturation, tenderness, water-holding capacity.**

I. Introduction

Juiciness is an important meat quality trait that contributes to the consumer's perception of overall acceptability including tenderness and juiciness. It is proposed that water-holding capacity is an important determinant of juiciness and tenderness and thus is a key measurement trait.

In pork, it is well-known that high muscle temperatures in the pre-rigor period will stimulate glycolysis, increase the rate of pH fall and can lead to protein denaturation of sarcoplasmic and myofibrillar proteins in the muscle cell. This results in unacceptably pale colour and high levels of water loss/exudate [1]. It is postulated that this may also occur in beef and lamb carcasses. Pale, watery meat in muscles of the beef hindleg have been reported [2] but no biochemical/protein denaturation studies have been conducted.

Superstretching of muscles or muscle strips should achieve minimal to no overlap between actin and myosin within the myofibrillar lattice structure. Myosin bound to actin is less susceptible to denaturation compared to unbound myosin. Thus according to [3], the rate of onset of rigor mortis and the subsequent degree of overlap between actin and myosin may determine the extent of myosin denaturation and thus the effect of temperature and pH fall on subsequent protein denaturation.

The aim was to determine the effect of temperature and muscle stretching on tenderness and water-holding capacity and how this is influenced by protein denaturation in four different hind-leg muscles in the sheep.

II. MATERIALS AND METHODS

The experiment was designed as a 2x2x2 with two electrical stimulation treatments (+/-), two stretch treatments (+/-) and two pre-rigor

temperature treatments (37°C vs 2°C). The experiment was conducted using 4 slaughter times and 48 lambs with each slaughter involving one slaughter day followed by 3 days in the laboratory isolating myofibrils and measuring tenderness and other parameters. The carcasses were randomly allocated to electrical stimulation and temperature treatments within slaughter days and the sides were randomly allocated to +/- stretch treatment within a carcass.

Lambs were slaughtered conventionally and the +/- electrical stimulation treatment applied. Low voltage electrical stimulation was applied at 1 min. post-slaughter using a rectal probe and stick wound clip. A square bipolar wave form was applied, providing 157 mA peak to peak (28-33V) with a frequency of 14 Hz and for a duration of 60 sec. The carcass was then suspended from the tail-bone. Each carcass had one side super-stretched (leg pulled down tightly and tied to ribs) and the other non-stretched (loosely held by a chain) and then a temperature conditioning treatment applied. The temperature treatments were (i) High temperature (37°C), carcass placed in 37°C vat for 4.5 hrs and then placed in chiller at 2°C, (ii) Low temperature (2°C), carcass placed into chiller at 2°C. The decline in pH and temperature was measured at 0.5, 1, 2, 3, 4 and 5 hrs post-slaughter in the *longissimus thoracis* at the 12th rib, using a portable pH meter and temperature probe.

At 24 hr post-slaughter, the muscles *gluteus medius* (GM), *rectus femoris* (RF), *semimembranosus* (SM) and *semitendinosus* (ST) were removed from each side of each carcass. The GM and SM was cut in half and each piece randomly allocated to 0 or 7 days of ageing. Samples destined for 7 days ageing were weighed into the bag, vacuum packed and stored at 2°C for the duration. The following measurements were conducted on all muscles after 0 days ageing; Minolta colour (L*a*b*, only L* is reported here) of muscle surface after 30 min. bloom, ultimate pH, surface exudate 10 min. after exposing surface, Warner-Bratzler peak shear force (tenderness) and cooking loss, myofibril purification, myofibrillar and sarcoplasmic protein solubility. A 2 x 1 cm^3 sample was removed from each muscles and frozen at -20°C for subsequent measurement of sarcomere length using laser diffraction.

After 7 days of ageing in a vacuum bag, the GM and SM samples were removed from the bags, the purge measured, samples removed for sarcoplasmic protein solubility and the samples cooked for measurement of water loss during cooking and Warner-Bratzler peak shear force. All methods are described in [1] except tenderness is described in [3].

Data was analysed using the ANOVA function in Genstat.

III. RESULTS AND DISCUSSION

A. Effect of Electrical Stimulation

Electrical stimulation had little effect on most of the meat quality traits measured ($P>0.05$) except for pH fall post-slaughter and thus electrical stimulation effects are only presented in the pH section below.

B. Sarcomere and muscle length

The +stretch treatment resulted in longer muscle length in the GM (~3 cm), SM (~4 cm) and ST (~6 cm) and also longer sarcomeres (~0.9-1.0 μm) in all three muscles compared to the -stretch treatment (Table 1; $P<0.001$ for all). Temperature appeared to have little effect on the muscle length or sarcomere length of the SM, ST or GM with one exception. For the ST, there was a bigger difference in sarcomere length between - and +stretch for the 2°C temperature treatment then for the 37°C treatment (interaction, $P=0.008$). The +stretch treatment had no effect on the muscle length of the RF muscle ($P>0.05$) but the sarcomere length of +stretch RF muscles was about 0.9 μm shorter then -stretch muscles. This indicates that the RF muscle is normally stretched by Achilles suspension and was free to shorten. In addition, the RF muscles held at 2°C were 0.3 μm shorter then those held at 37°C. This indicates that because the RF muscle was free to shorten, the 37°C treatment resulted in 'heat'-shortening.

C. Temperature and pH decline rates

The rate of temperature fall in the loin muscle was very fast in the cold treatment and reached <10^{o}C by 5 hrs post-slaughter (Figure 1). The warm treatment remained high until 5 hrs post-slaughter at which time the carcasses were placed in the chiller.

The electrically stimulated carcasses had a much lower pH at 0.5 and 1 hr post-slaughter (~0.5-0.6 units; Figure 2) compared to the non-stimulated carcasses (P<0.001). Thereafter, for the stimulated carcasses, the pH was similar between the 2^{o}C and 37^{o}C treatments (P>0.05) but for the non-stimulated carcasses, the pH was 0.4-0.6 units lower for the 37^{o}C treatment compared to the 2^{o}C treatment (Temp x es interaction at 2-5 hrs post-slaughter; (<0.10 for all). Thus the cold treatment, non-stimulated carcasses had a much slower rate of pH fall than the other three treatments and the conditions in the LTl muscle were conducive to cold-shortening occurring (temp<10^{o}C while pH >6.0).

The 37^{o}C treated GM muscle had a higher ultimate pH then the 2^{o}C treated GM muscle (5.74 vs 5.66; P<0.01) and all other muscles had a similar ultimate pH between treatments (~5.80; P>0.05).

D. Tenderness

For the Warner-Bratzler values, there was an interaction between stretch and temperature treatments at both one and eight days post-slaughter post-slaughter for all muscles. For the -stretch treatment, the 37^{o}C treatment produced significantly tougher meat in the GM and SM at 1 and 8 days post-slaughter compared to the 2^{o}C. In contrast, for the stretched muscles (+stretch for the GM,SM, ST and -stretch for the RF), the 37^{o}C treatment produced significantly more tender meat in all muscles at one day post-slaughter compared to the 2^{o}C treatment.

E. Water Loss

For both muscle types at one day post-slaughter, the slowly chilled muscles had a higher surface exudate (~20 mg higher), a higher cook loss (~6% higher) and a higher purge (~2% higher) than the cold treatment (P<0.001 for all). At eight days post-slaughter for the GM and SM muscles, the slowly chilled muscles also had a higher cooking loss relative to the fast chilled muscles (P>0.05). Thus the higher temperature treatment clearly reduced the water-holding capacity of the muscle but in the case of cooking loss, this effect appears to be removed by ageing the muscle.

All of the stretched muscles (+ stretch treatment for the GM, SM and ST and -stretch for the RF) had a lower surface exudate (P<0.05 for all), lower purge (P<0.01 for both) and a lower cooking loss at both one and eight days post-slaughter (P<0.05).

There was an interaction, or a trend for an interaction, between stretch and temperature treatment for the SM and ST surface exudate and the SM purge (P<0.10 for all). For the fast chilled carcasses, there was no difference in purge or exudate between the stretch treatments (P>0.05) whereas for the slowly chilled muscles, the +stretch muscles had lower exudate and purge (P<0.05).

F. Colour

For the 37^{o}C treated samples, the lightness (L*) was similar between the stretch and non-stretch (P>0.05) whereas the 2^{o}C stretched muscles (-stretch for the RF) was darker then for the 2^{o}C non-stretched muscles (temp x stretch interaction, P<0.05).

The rapidly chilled, stretched muscles had a darker surface (lower L*) than the rapidly chilled non-stretched samples. This was not the case for slowly chilled muscle where the stretch and non-stretch samples were lighter than the rapidly chilled muscles but similar in lightness to each other.

G. Protein denaturation

For all muscles except for the RF, the 37°C treatment reduced sarcoplasmic protein solubility compared to the 2°C treatment at both 1 and 8 days post-slaughter ($P<0.05$ for all). For the ST muscle, 2°C treated samples did not show any affect of stretch but for 37°C treatment, +stretch had lower sarcoplasmic protein solubility than for -stretch samples (temp x stretch interaction, $P=0.095$). This interaction was also evident in the myofibrillar ATPase activity for several muscles. For the SM and ST muscles, 2°C treated samples did not show any affect of stretch but for 37°C treatment, +stretch had lower myofibrillar ATPase activity than for -stretch samples (temp x stretch interaction, $P<0.01$ for all). For the GM muscle, 37°C samples had lower myofibrillar ATPase activity than the 2°C samples ($P<0.001$) and the +stretch muscles had lower myofibrillar ATPase activity than the -stretch samples ($P=0.003$). Thus the high temperature treatment clearly resulted in increased muscle protein denaturation. In addition, the stretching of the muscles generally resulted in increased protein denaturation, particularly of the myosin head and particularly when combined with the high temperature treatment.

IV. DISCUSSION AND CONCLUSION

The stretch treatment was successful in achieving a significant lengthening of the muscle, muscle fibres and sarcomeres in the GM, SM and ST muscles, as indicated by muscle and sarcomere length. The opposite was achieved by the stretch treatment in the RF muscle due to its anatomical location in the carcass.

There was no evidence of cold-shortening or heat-shortening in the SM, GM and ST muscles, as indicated by sarcomere length. There was evidence of 'heat-toughening' in the high temperature treatment in the GM, SM and RF non-stretched muscles (+stretch in the case of RF). It would appear that the stretch treatment applied overcame the heat-toughening caused by the 37°C treatment evident in the non-stretched muscles.

Thus muscles exposed to high temperature conditions in the sheep carcass will demonstrate heat-toughening which can be alleviated by stretching the muscles. The increased toughness in the 37°C muscles in the no stretch treatments was associated with more water loss (purge and surface exudate) but less myosin denaturation (myofibrillar ATPase activity) compared to the stretch treatments.

Thus rapid pH fall and high muscle temperature did result in increased muscle protein denaturation and paler, more watery lamb meat. But the effect of these conditions on the tenderness varied with the stretch treatment. When the lamb side was stretched, high temperature and rapid pH fall resulted in more tender meat. In the no stretch treatment ('normal' for industry practice), the rapid pH fall and high muscle temperature resulted in 'heat-toughening'. The mechanism driving the 'heat-toughening' is not clear as there was no evidence of 'heat-shortening' or of increased muscle protein denaturation. When the muscle was stretched so that minimal overlap would have occurred between myosin and actin, the myosin denaturation increased at the high temperature. But the water-holding capacity of the muscle was reduced, not increased relative to the no stretch treamtent.

In conclusion, heat-toughening occurred in non-stretched lamb muscle subjected to 37°C for 4.5 hrs. The high temperature treatment resulted in excessive water loss from the muscles and high levels of muscle protein denaturation. As the high temperature treatment conditions are unlikely to be encountered in an industry environment for either beef or lamb muscles, it is important to investigate the occurrence of heat-shortening under more moderate temperature regimes.

ACKNOWLEDGEMENT

Funding provided by Meat and Livestock Australia is gratefully acknowledged.

REFERENCES

[1] R. D. Warner, R. G. Kauffman, and M. L. Greaser, Muscle protein changes *post mortem* in relation to pork quality traits, Meat Science, 45(3) (1997) 339-352.

[2] P. V. Tarrant and C. Mothersill, Glycolysis and associated changes in beef carcasses, Journal of Science in Food and Agriculture, 28 (1977) 739-749.

[3] P. E. Bouton, P. V. Harris, and W. R. Shorthose, Effect of ultimate pH upon the water-holding capacity and tenderness of mutton, Journal of Food Science, 37 (1971) 358-360.

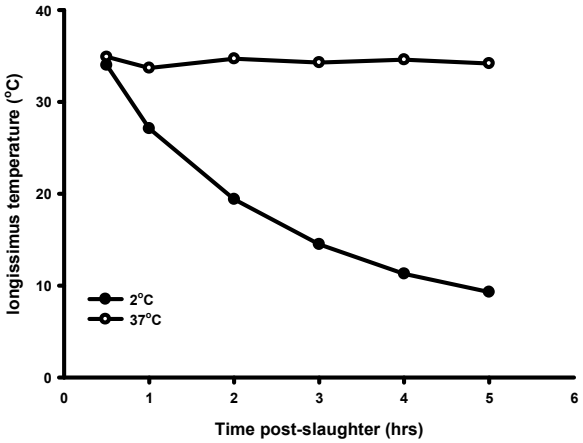

Figure 1: Effect of holding temperature (2°C vs 37°C) on the decline in temperature post-slaughter in the *longissimus thoracis.*

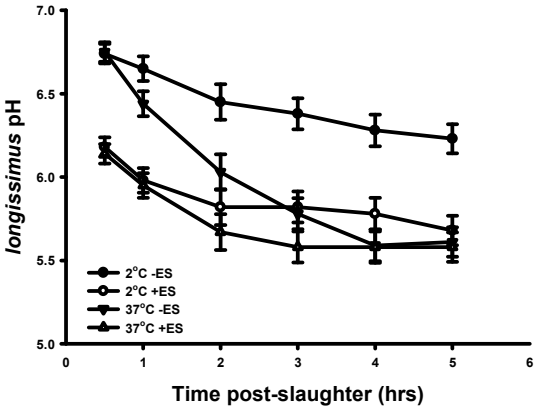

Figure 2: Effect of electrical stimulation (-ES vs +ES) and holding temperature (2°C vs 37°C) on the decline in pH post-slaughter in the *longissimus thoracis.*

Table 1: Effect of pre-rigor temperature (2 °C vs. 37°C) and stretch _(-stretch vs +stretch) on quality traits for the muscles gluteus medius (GM), rectus femoris (RF), semimembranosus (SM) and semitendinosus (ST).

	Temperature 2oC		Temperature 37oC	
	-stretch	+stretch	-stretch	+stretch
Warner-Bratzler- 1 day post-slaughter (kg)				
GM	5.14b	4.76bc	7.72a	3.98c
RF	5.36	5.40	4.45	5.51
SM	5.25b	4.87bc	6.81a	4.05c
ST	6.41a	5.62b	5.82b	3.94c
Warner-Bratzler- 8 days post-slaughter (kg)				
GM	3.22b	3.43b	6.26a	2.90b
SM	3.01b	3.46b	4.84a	3.10b
Surface Exudate (mg)				
GM	13.4c	10.6d	29.9a	25.4b
RF	10.4d	16.9c	22.1b	28.9a
SM	13.4c	12.2c	39.2a	28.1b
ST	14.2c	11.3c	40.2a	27.0b
Purge (%)				
GM	3.88c	2.48d	6.15a	5.14b
SM	3.28c	2.63c	6.08a	4.48b
Cooking loss - 1 day post-slaughter (%)				
GM	29.8c	26.9c	35.7a	34.2b
RF	32.2c	35.6b	39.1a	40.0a
SM	30.1c	27.5c	37.2a	34.2b
ST	30.5c	26.5d	38.2a	34.6b
Cooking loss - 8 days post-slaughter (%)				
GM	33.9b	32.4b	35.8a	33.2b
SM	33.6b	32.6b	35.5a	32.9b
Sarcoplasmic protein solubility (mg/100g)				
GM	51.0a	49.8a	44.6b	44.9b
RF	39.2	39.7	37.0	36.7
SM	51.8a	52.6a	45.4b	46b
ST	45.8a	45.6a	40.3ab	38.3b
Myofibrillar ATPase activity (μmol/min/mg protein)				
GM	0.130a	0.113ab	0.109b	0.073c
RF	0.112	0.116	0.105	0.117
SM	0.136a	0.131a	0.125a	0.087b
ST	0.134a	0.124a	0.122a	0.066b
Surface lightness (L*)				
GM	34.9b	33.8b	36.7a	36.8a
RF	35.8b	37.9a	38.6a	38.0a
SM	33.5b	32.2c	35.5a	35.7a
ST	39.7b	37.9c	41.6a	41.8a
Sarcomere length (μm)				
GM	2.01a	3.01b	1.92a	2.71b
RF	3.06a	2.15c	2.76b	1.86d
SM	2.09b	3.03a	2.05b	2.84a
ST	2.20b	3.35a	2.20b	3.21a

abcd Means within rows with different superscripts differ at $P<0.05$.