Effects of pH Early Postmortem on Meat Quality in Beef Longissimus

I. H. Hwang* and J. M. Thompson

Co-operative Research Centre for the Cattle and Beef Industries, University of New England, Armidale, N S W, 2351, Australia

ABSTRACT : The effects of type (high and low voltages) and time (3, 40 and 60 min postmortem) of stimulation on drip loss and meat color at 24 h post-mortem were determined on *M. longissimus dorsi* of 38 crossbred steers and heifers. In addition, the effect of pH early postmortem (70 min postmortem) on the rate and extend of meat tenderization was examined. Either high or low voltage stimulation at 3 min showed a tendency for faster pH decline ($p=0.052$) and higher drip loss ($p=0.08$), and improved the color dimensions of L*, a* and b* ($p<0.01$), compared to stimulation at 40 min. This was equivalent to approximately one unit of an AUS-MEAT color chip. On the other hand, although there were significant differences in pH decline between high voltage stimulation at 40 and 60 min, and between low voltage stimulation at 40 min and control sides, drip loss and meat color did not differ significantly ($p>0.05$). The results suggested that early application of stimulation, regardless of type of stimulation, improved overall meat color at 24 h postmortem through its effect on faster glycolysing rate. However, if the pH decline was moderate, the benefit of electrical stimulation on meat color was not apparent. An intermediate pH decline resulted in the lowest shear force. Due to differential ageing rates the optimum pH at 70 min postmortem increased with ageing time from 5.96, 6.07, 6.12 and 6.14 for 1, 3, 7 and 14 days postmortem, respectively. This implied that a small difference in the rate of pH decline was important, especially carcasses stimulated for very early postmortem, and the optimum rate of pH decline varied with intended ageing period. The study suggests that the beneficial or adverse effects of electrical stimulation on drip loss, meat color and tenderness is determined by the rate of pH decline, rather than by stimulation treatment and time of application *per se*. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 8 : 1218-1223)***

Key Words : Beef, pH, Meat Color, Tenderness

INTRODUCTION

The rate of pH decline in electrically stimulated carcasses is determined by many factors including current, voltage and the time of application (Chrystall and Devine, 1985). In practice, low voltage stimulation is applied immediately after slaughter, whilst high voltage stimulation is applied anywhere from 20 minutes to one hour after slaughter. There have been a number of attempts (eg. McKeith, et al., 1980; Eikelenboom et al., 1985; Aalhus et al., 1994) to determine the efficiency of high and low voltage systems in the improvement of meat quality. However, many of these comparisons were confounded by differences in rigor temperature due largely to differences in time of application.

Apart from improving meat tenderness, electrical stimulation has been shown to improve initial meat color (Pommier, 1992; Powell et al., 1996), although its stability during extended retail display has been questioned by several researchers (Unruh et al., 1986; Geesink et al., 1994). The increased denaturation of water-binding myofibrillar proteins (Swatland, 1994) and (or) early attainment of the average iso-electric point in electrically stimulated muscle (Lawrie, 1991) have been proposed as mechanisms associated with the improved meat color. McKeith et al. (1980) showed that high voltage stimulation improved subjective lean-color scores, compared with low voltage stimulation. However, it has been suggested that pH/temperature profile during the onset of rigor is a more significant determinant of meat color early postmortem because meat color is significantly affected by the stability of myoglobin molecules and enzymes involved in color development (D. Gutzke, Pers. com.).

This experiment was conducted to investigate the effects of high and low voltage stimulation, applied at the same or different times postmortem on the rate of pH decline, meat color and drip loss at 24 h, which is the time when chiller assessment of carcass quality is generally performed. In addition, the effect of the rate of pH decline early postmortem on meat tenderness and tenderization was examined. Preliminary data from this study was presented at the 8th Annual Congress of Asian Australasian Association of Animal Production Society.

MATERIALS AND METHODS

Animals and treatments

The animals comprised 38 pasture-fed steers and heifers, which were the progeny of 3 sire breeds (Angus, Brahman and Piedmontese) crossed with Brahman×Hereford crossbred cows. The animals were transported from Glen Innes Research Station to the Food Science Australia, Meat Laboratory in Brisbane and kept on pasture for at least a

* Corresponding Author: I. H. Hwang, National Livestock Research Institute RDA, 564 Omokchun-dong, Suwon 441-350, Korea, Tel: +82-31-290-1702, Fax: +82-31-290-1697, E-mail: ihhwang@rda.go.kr

Received April 30, 2002; Accepted April 23, 2003

week prior to slaughter. Mean age and live weight were 660±21 days and 444±45 kg, respectively.

Animals were slaughtered in groups of 6 or 8 each day over six slaughter days, with animals within breed and sex categories randomized across treatments and days. All animals were stunned using a captive bolt. Eighteen bodies were stimulated using either a high (HV3), or low (LV3) voltage system applied to the whole body 3 min postmortem for 40 seconds immediately after bleeding, via electrodes inserted in the nostril and rectum, with both legs shackled. The remaining 20 animals were dressed, halved and the left sides stimulated at approximately 40 min postmortem by either high (HV40), or low (LV40) voltage. The corresponding right sides of the HV40 treatment were stimulated with high voltage at 60 mins postmortem (HV60), whilst the corresponding right sides of LV40 treatment served as control sides (Cont). Stimulation at 40 and 60 mins postmortem was applied for 55 seconds via 2 multi-point electrode probes inserted into the muscles at the proximal end of the Achilles tendon and lateral aspect of the scapula. Low voltage (70 peak volts) stimulation comprised an uni-directional square wave pulses of 7 ms width, 14.3 pulses per second and 1 ampere. High voltage (800 RMS of continuous alternating polarity) stimulation comprised bi-directional half-sinusoidal pulses of 10 ms width, 14.3 pulses per second with 7.2 amperes. Carcass sides were placed in a 1°C chiller approximately 50 mins postmortem, with the exception of the sides receiving the HV60 treatment, which were placed in the same chiller immediately after stimulation.

Sampling and measurements

Following removal of the hide, temperature and pH were monitored using a portable needle-tipped combination electrode (ORION®, USA). Measurements were recorded approximately every 15 mins in the caudal end of *M. longissimus lumborum* until the muscle was judged to have reached ultimate pH. An additional measurement was taken the following day, approximately 24 h postmortem.

The day following slaughter the striploin (from the 5th thoracic vertebrae to the last lumbar vertebrae) was removed from each carcass side, and trimmed of all epimysium. Four blocks of approximately 250 g (ca. 9×7×4 cm) were cut from the cranial end for objective laboratory measurements. These blocks were vacuum packed and randomly assigned to one of the four ageing periods (1, 3, 7 and 14 days at 1°C) prior to being frozen at -20°C until analysis. A modified Warner-Bratzler shear force was measured using the method described by Bouton et al. (1971) and Bouton and Harris (1972). In order to minimise any ageing occurring between the frozen state and cooking, the blocks were cooked from the frozen state for 60 min at 70°C in a water bath.

Objective color dimensions (L*, lightness; a*, redness; b*, yellowness) were assessed on the cranial portion of the loin at 24 h postmortem using a Minolta CR-300 Chroma Meter. The measurement was the average of three readings over the muscle surface after it had been exposed to air for 60 min at 1°C.

Drip loss was measured as the weight loss in an 85 g portion of muscle adjacent to the color samples and hung in a plastic bag at 1°C for an additional 48 h (Taylor and Dant, 1971).

Experimental design and statistical analyses

The treatments enabled a main and two sub-experiments of factorial arrangement, to be overlaid on the data. Experiment 1 examined the interaction between type of stimulation (high or low voltage) and the time of stimulation (3 or 40 min), on a between carcass basis. Experiment 2 compared time of application of high voltage stimulation, at either 40 and 60 min postmortem, on a within carcass basis. Experiment 3 compared low voltage stimulation at 40 min postmortem with the corresponding non-stimulated control sides, on a within carcass basis.

The decline in pH as a function of time after stunning was modelled (SAS, 1997) using the exponential function: $pH_t=pH_u+(pH_i-pH_u)e^{-kt}$, where pH_t=pH at time t, pH_u= ultimate pH, pH_i=initial pH, k=exponential constant, t= time in fractions of a day (Hwang and Thompson, 2001b).

In Experiment 1, pH and drip loss were analyzed using a mixed model, which contained type and time of stimulation, sire breed, sex and the interaction between time and type of stimulation and animal as a random effect. Color dimensions were analysed using a multivariate model (Gilmour, 1992), where L*, a* and b* were analysed simultaneously. The final model contained the same terms for pH and drip loss. Models for Experiments 2 and 3 were similar to those of experiment 1, except that type of stimulation and interaction between type and time of stimulation for experiment 2, and time of stimulation and interaction between type and time of stimulation for experiment 3 were excluded from the model. WB-shear force as a function of pH at 70 min postmortem was estimated using a mixed model. The initial model contained fixed effects (breed and sex), covariates (linear and curvilinear effects for pH and temperature at 70 min postmortem), first and second order interactions between these terms, and a random effect for animal. Non-significant interactions and covariates ($p>0.05$) were sequentially excluded from the models. Breed and sex was retained in the models, but effects are not reported in this study.

RESULTS AND DISCUSSION

pH decline and drip loss

Table 1. Predicted means, F ratios and significance levels (adjusted for breed and sex) for pH at 70 min and 24 h postmortem (pH_{70} and pH_{24}), temperature at pH 6.0 ($Temp_{6.0}$), drip loss and L*, a* and b* values at 24 h postmortem

	Treatment[1]				Av. SE	F ratio		
						Time[2]	Stim[3]	Time×Stim
Experiment 1	HV3	LV3	HV40	LV40		(df 1,31)[4]	(df 1,31)	(df 1,31)
pH_{70}	5.73	5.80	5.89	5.94	0.07	$4.08^{P=0.052}$	0.74	0.02
pH_{24}	5.48	5.44	5.40	5.46	0.04	0.5	0.14	1.59
$Temp_{6.0}$ (°C)	37.6	36.8	36.1	35.1	1.16	0.54	1.87	0.01
Drip loss (%)	1.2	1.07	0.86	0.84	0.15	$3.42^{p=0.07}$	0.25	0.13
L*	39.4	43.0	38.4	37.7	0.72	19.6^{***}	3.8	8.8^{**}
a*	23.7	24.6	22.0	21.2	0.59	20.2^{***}	0.0	2.1
b*	11.3	12.4	10.4	5.6	0.41	20.9^{***}	0.2	5.3^{*}
Experiment 2	HV40		HV60			(df 1,15)		
pH_{70}	5.89		6.24		0.07	13.07^{**}	na	na
pH_{24}	5.40		5.39		0.03	0.09	na	na
$Temp_{6.0}$ (°C)	35.9		33.3		0.74	6.32^{*}	na	na
Drip loss (%)	0.84		1.02		0.16	0.06	na	na
L*	37.4		36.7		0.55	0.82	na	na
a*	21.1		21.3		0.37	0.06	na	na
b*	9.5		9.3		0.38	0.17	na	na
Experiment 3	LV40		Cont				(df 1,15)	
pH_{70}	5.94		6.68		0.06	na	77.5^{***}	na
pH_{24}	5.47		5.53		0.03	na	1.63	na
$Temp_{6.0}$ (°C)	35.3		20.7		2.00	na	25.9^{***}	na
Drip loss (%)	0.82		0.78		0.12	na	0.04	na
L*	38.3		37.8		0.60	na	0.2	na
a*	21.9		22.3		0.51	na	0.05	na
b*	10.3		10.7		0.41	na	0.001	na

[1] HV3, HV40 and HV60: high voltage stimulation applied at 3, 40 and 60 min postmortem, respectively; LV3 and LV40: low voltage stimulation applied at 3 and 40 min postmortem, respectively; Cont: unstimulated control; [2] Time of stimulation; [3] Type of stimulation L*: Lightness, a*: redness, b*: yellowness; [4] Degree of freedom (numerator, denominator); $^{***}p < 0.001$; $^{**}p < 0.01$; $^{*}p < 0.05$; na: not applicable.

When either high or low voltage stimulation was applied at 3 min, there was a tendency for a faster decline in pH early postmortem (e.g., 70 min) than either high or low stimulation applied at 40 min (p=0.052, Table 1). Time of stimulation had no effect on ultimate pH. Similarly, HV40 treatment resulted in a faster rate of pH decline compared with the HV60 treatment ($p<0.01$), which was consistent with the results of Kastner et al. (1993). This indicated that the magnitude of pH decline in electrically stimulated carcass was largely a function of the time of application. The trend for an increased drip loss in carcasses stimulated at 3 min (p=0.07, Table 1) supported the suggestion that a rapid rate of pH fall in electrically stimulated carcass increased the risk of protein denaturation (Offer et al., 1989; den Hertog-Meischke et al., 1997). However, it was only the early postmortem period where stimulation time affected drip loss, as there was no difference in drip loss when high voltage stimulation was applied at either 40 or 60 min. These results were similar to those of Taylor and Tantikov (1992) and Taylor and Martoccia (1995) in pork. The current results indicated that the time of stimulation, which determined the rate of pH decline, had a more significant influence on drip loss rather than stimulation treatment *per se*.

Table 2. F ratios and significance levels for the multivariate analysis of L*, a* and b* color dimensions (adjusted for breed and sex)

	df[1]	F ratio
Experiment 1		
Time	3/29	8.73^{**}
Stimulation	3/29	1.43
Time x Stimulation	3/29	3.24^{*}
Experiment 2		
Time	3/13	0.6
Experiment 3		
Stimulation	3/13	0.08

[1] Degree of freedom (numerator/denominator); $^{*}p<0.05$; $^{**}p<0.01$.

Objective meat color

In experiment 1, the multivariate analysis showed a significant interaction between type and time of stimulation ($p<0.05$, Table 2). The univariate analyses in Table 1 showed that the significance of the interaction was due largely to changes in L* and b* dimensions, with little effect on the a* dimension. Stimulation at 3 min resulted in an increase in the L* dimension relative to stimulation at 40 min, with a greater increase in LV3 treatment. For the b* dimension the interaction was one of magnitude with the LV40 treatment having the lowest reading. Reasons for these interactions were not clear, because pH decline

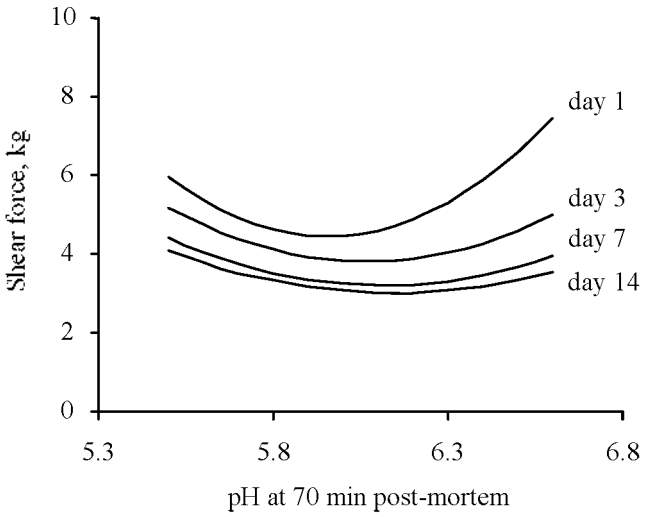

Figure 1. Predicted shear force for electrically stimulated carcasses as a function of pH at 70 minutes postmortem at 1, 3, 7 and 14 days aging at 1°C (adjusted for breed and sex). The model explained 56% of variations. 1. Predicted shear force for electrically stimulated carcasses as a function of pH at 70 minutes postmortem at 1, 3, 7 and 14 days aging at 1°C (adjusted for breed and sex). The model explained 56% of variations.

appeared to be largely a function of time of stimulation. Mikami et al. (1993) reported a greater denaturation of water binding myofibrillar proteins in electrically stimulated carcass, which was related to a lighter muscle color (Pearson and Dutson, 1985). However, in the current study, even though the rate of pH decline and drip loss were higher when stimulation was applied earlier after stunning, only the LV3 treatment showed a higher L* value. Our results do not support the study of McKeith et al. (1980), who reported that high voltage, irrespective of time of application and length of stimulation, resulted in higher lean-color scores compared with low voltage stimulation. Even in the presence of interactions, the main effects for time of stimulation on color dimensions were highly significant ($p<0.001$, Table 1), with stimulation at 3 min resulting in increased L*, a* and b* dimensions. When the predicted values were compared with L*, a* and b* values of AUS-MEAT color chips (Anon., 1997), the difference was equivalent to approximately one color unit in each color dimension. Because this coincided with a faster pH decline and higher drip loss, it appeared to be a consequence of protein denaturation, and (or) earlier opening up of the muscle structure as proposed by Lawrie (1991). This was consistent with the results of Unruh et al. (1986) and Pommier (1992) in studies where low voltage stimulation was applied soon after stunning. This suggested that early application of stimulation improved meat color at 24 h as a consequence of fast pH decline.

On the other hand, in experiments 2 and 3, the color dimensions at 24 h were similar, although there were significant differences in pH decline between HV40 and HV60 treatments, and between LV40 and control. This does not agree with previous reports showing that electrical stimulation improved meat color (Savell et al., 1978; Aalhus et al., 1992). However, it has been shown that the benefit of electrical stimulation on color was diminished (Salm et al., 1981) or was undetected (Powell, 1991) at 24 after slaughter. With considering the previous studies, it was considered that if the pH decline was relatively slow, its effect on meat color was not detectable at 24 h when the chiller assessment of carcass quality is performed. In addition, the contradictory results of previous studies may be due to effects of the rate of pH decline as well, rather than simply the effects of electrical stimulation treatment and type of stimulation.

Effects of pH early postmortem on shear force and ageing rate

This report was intended to highlight meat color and drip loss, as tenderness aspect previously were reported (Hwang and Thompson, 2001a). However, it was of particular interest to reanalyze pooled data as a response surface, since large overlaps in pH decline and WB-shear force existed between treatments. This analysis was also performed to confirm the conclusion reached by our early study (Hwang and Thompson, 2001b). In this study, an adverse effect of rapid glycolysis on meat tenderness appeared to be associated with the early reduction in μ-calpain, whilst the levels of calpastatin remained relatively high and that was related to the rate of pH decline. Under the current experimental conditions, 62% and 94% of stimulated carcasses reached a pH of lower than 6.0 at 70 minutes and 2 h postmortem respectively (data not shown), and the largest variation in pH decline occurred at 70 minutes postmortem. Figure 1 shows shear force as a function of pH at 70 minutes postmortem for electrically stimulated carcasses. The model, which explained 56% of the variations of shear force, included significant linear and curvilinear terms of pH at 70 minutes and their first and second order interactions. Non-stimulated carcasses were excluded from the modeling, because shear force apparently ranged far out of the population.

Figure 1 shows that, as ageing time increased, the points of minimum shear force moved toward a higher 70-min pH region due to the higher ageing rate when pH was higher at 70 minutes. The lowest shear force resulted from a 70-min pH of 5.96, 6.07, 6.12 and 6.14 at 70 minutes postmortem for day 1, 3, 7 and 14 aged meat, respectively. A lower ageing rate at the left portion of Figure 1 comprised mostly of sides from HV3 and LV3 treatments, and would be associated with a high rigor temperature (Pommier et al., 1987; Powell, 1991; Dransfield et al., 1992; O'Halloran et

al., 1997) and is a consistent with our early study (Hwang and Thompson, 2001b). Although there was an overlap, carcasses of HV40, LV40 and HV60 treatments were mainly overall on the intermediate to right section of the graph in Figure 1. On the basis of early studies (eg. Dransfield et al., 1992; Simmons et al., 1996; Hwang and Thompson, 2001b), it was thought that early exhaustion of μ-calpain was linked to a lack of tenderization for the left section of the graph. In contrast, carcasses situated on the right portion of the response surface showed a higher ageing rate even though meat was not as tender as that for an intermediate region after 14 days of ageing. pH/temperature profiles for the group with slow pH decline (eg. right region of the graph) showed approximately 33-35°C at a pH of 6.0 (Table 1), indicating that there was no particular cold shortening. This result suggests that the time when the tenderizing process is initiated has a direct effect on the rate of tenderization and ultimate tenderness.

Other studies showed that an intermediate pH decline (ca. 5.9–6.1 at 3 h postmortem) produced the most tender meat (Marsh et al., 1987; Smulders et al., 1990; Pike et al., 1993). However we failed to find any significant quadratic relationship between shear force and pH at 3 h or near 3 h postmortem. The result suggests that a small difference in pH decline early postmortem for electrically stimulated carcasses is a significant determinant of tenderness and tenderisation, since stimulated carcasses tend to enter rigor at a relatively high temperature. The detrimental effect of an excessive pH decline was evidenced by smaller decreases in shear force values as ageing time increased. Electrical stimulation improved tenderness, however the results suggests that in order to increase the efficiency of electrical stimulation excessive pH decline, via early application of stimulation needs to be avoided.

IMPLICATIONS

The current results indicate that early application of stimulation, irrespective of type of stimulation, improved meat color at 24 h, with fast pH decline and increased drip loss. However, the effect of electrical stimulation treatment on meat color and drip loss was not apparent if the glycolysing rate was moderate. Overall an intermediate rate of pH decline as assessed by the 70-min pH resulted in the lowest shear force, however due to differential ageing rates the optimum rate of pH decline increased with ageing time. This implies that a small difference in the rate of pH decline may be important, especially for very early postmortem glycolysis in electrically stimulated carcasses. These results suggest that the effect of electrical stimulation on meat color, drip loss and tenderness is a function of the rate of pH decline, rather than of the stimulation treatment *per se*.

ACKNOWLEDGEMENTS

Ms. H. Hearnshaw and Mr. F. D. Shaw are thanked for providing animals, technical assistance and advice.

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