EFFECTS OF ADDITIONAL ELECTRICAL STIMULATION AND PRE-RIGOR CONDITIONING TEMPERATURE ON AGEING POTENTIAL OF HOT-BONED BEEF MUSCLE

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Abstract – The objective of this study was to determine the impact of various *pre-rigor* holding temperatures along with accelerated pH decline rates due to additional electrical stimulation on the meat tenderness of *M. longissimus lumborum* from bulls that were electrically head-only stunned, immobilized and Halal slaughtered. Paired loins from 12 bulls were hot-boned within 40 min of slaughter, one side was immediately electrically stimulated (AES) and the other side was not stimulated (NES). The samples were then subjected to various holding temperatures (5°C, 15°C, 25°C and 35°C) for 3hrs and the rate of muscle pH decline, and various meat quality parameters were measured. AES did not result in faster pH decline, nor did the process influence cook loss, purge and drip loss (48hrs *post mortem*). However, after 14 days of ageing, drip loss was significantly decreased by AES. The 3hrs *pre-rigor* holding temperature accelerated pH decline, decreased shear force values, and lowered drip loss (at both 48hrs and 14 days *post mortem*). Overall, this study demonstrate that *pre-rigor* holding temperature (e.g. 25°C) alone or in combination with AES resulted in lower shear force values, and increased water holding capacity in bull beef samples.

Key Words – electrical stimulation, meat quality, *pre-rigor* holding temperature, proteolysis.

I. INTRODUCTION

Meat tenderness is one of the key quality attributes affecting consumers' eating satisfaction and repeat purchasing decision. Different *pre-rigor* environments generated by the application of electrical stimulation and/or *pre-rigor* chilling conditions influence the rate of glycolysis and subsequent pH decline in *post mortem* muscles [1]. The complex interaction of pH and temperature decline in *pre-rigor* muscle has an important role in meat tenderization by modulating proteolytic enzyme activity [1]. Recently [2, 3], we have demonstrated that various *pre-rigor* holding temperatures (especially 25 or 35°C) along with accelerated pH decline rates produced by low voltage electric stimulation (LVES) improved the tenderness and reduced cook loss of bull *M. longissimus lumborum*. The treatments resulted in higher µ-Calpain activity (autolysis) and moreover, myofibrillar proteins (desmin and troponin-T) and sHSP degradation were highest for LVES samples. LVES combined with the 3hrs *pre-rigor* holding temperature (for ES-25 and ES-35 samples) resulted in no cold shortening (or heat induced shortening) even though the samples were aged at 1°C. Based on the above findings [2, 3], it can be implied that there is an optimal condition (i.e. combination of LVES with 3hrs *pre-rigor* holding temperature at 25 or 35°C) for maximizing the ageing-potential for bull beef. However, because these results were obtained from bulls which were stunned using captive bolt (carried out to avoid any electrical effects before stimulation), the outcomes needed to be verified using animals that are head-only electrically stunned pre-slaughter. Thus, the objective of this study is to determine the impact of additional electrical stimulation (AES) and various *pre-rigor* holding temperature (for 3hrs) on the ageing-potential of hot boned muscles from bulls that are head-only electrically stunned pre-slaughter.

II. MATERIALS AND METHODS

Raw materials and processing

A total of 12 cattle (around 24-month-old bulls) were slaughtered at a commercial New Zealand meat plant over three slaughter days. Bulls in this study were electrically stunned for Halal kill (frequency = 50 Hz, pulse width = 3.5 milliseconds, peak voltage = 583 volts) and the carcasses electrically immobilized following exsanguination. Both loins (M. longissimus lumborum; LL) from

the 12 beef carcasses were hot-boned within 40 min *post mortem*. In this study, two main treatment effects i.e., additional electrical stimulation (AES/NES) and *pre-rigor* holding temperature (at 5°C, 15°C, 25°C and 35°C for 3hrs) and their interactions were tested giving a total of 8 different treatment combinations. Initial pH (pH_{40min}) was recorded and the LL from one side of the carcass was immediately subject to low voltage electrically stimulation (AES) for 30 seconds after boning (frequency = 13.3 Hz, pulse width = 5.4 milliseconds, peak voltage = 104 volts) and the LL from the other side of the carcass was not electrically stimulated (NES). After the AES, pH was recorded once again. Immediately after stimulation or non-stimulation, approximately 10 g of muscle was removed, snap frozen using liquid nitrogen and stored at -80°C for initial biochemical analyses. The loins were then divided into four different sub-samples, placed in plastic bags and randomly submerged in either 5°C, 15°C, 25°C or 35°C water baths for 3hrs (*pre-rigor* holding temperatures). After 3 hours had elapsed, the meat/muscle samples were removed from the plastic bags; their pH measured, and further sampled for biochemical analysis. The samples were transferred to the AgResearch laboratory where they were vacuum packed and aged at 1°C for 24, 48hrs, and 14 days *post mortem*. Muscle samples for various analyses were taken at 48hrs and 14 days *post mortem*, respectively.

pH

pH of the loin samples was measured in duplicate by inserting a calibrated pH probe (Hanna HI99163 pH meter with a FC232D combined pH/ temperature probe, HANNA instruments, Rhode Island, USA) directly into the muscle at 40 min (before and after stimulation), 3hrs, 6hrs, 24hrs, 48hrs and 2 weeks *post mortem*, respectively.

Shear force

The loin cuts were cooked in a water bath set at 99°C to an internal temperature of 75°C (measured by 12 channel Digisense Thermocouple Thermometer). After cooling, 10 mm x 10 mm cross section samples were cut and sheared using MIRINZ Tenderometer. Ten replicates were measured for each sample. The results were expressed as shear force (kgF) [4].

Purge and drip loss (*water holding capacity*)

The loin sections were weighed prior to vacuum-packaging to obtain initial weight for the purge loss measurement. After the assigned storage time, the samples were removed from the vacuum bags, patted dry on paper towels and reweighed (final weight) to determine purge loss as the difference between initial weight and final weight expressed as %. Drip loss was measured after each assigned storage (48hrs and 14 days) following the procedure of Honikel [5].

Western blot

Whole muscle protein extraction, gel sample preparation and Western blotting (desmin, troponin-T, μ calpain, sHSP (αβ-crystallin and HSP20) were performed as described previously [4].

Data analysis

All statistical analysis was performed using Genstat 16th edition [6]. The pH fall was analyzed using ANOVA where side within animal was the blocking variable and the treatment variables were temperature, electric stimulation, time and all possible 2- and 3-way interactions. Firstly data was analyzed excluding pHu using ANOVA. The shear force, drip and purge loss (for 48hrs and 14 days) data were the dependent variables. For the above mentioned analysis, side within animal was included as a blocking variable and the treatment variables were temperature (5°C, 15°C, 25°C and 35°C) and additional low voltage electrical stimulation and their interaction. Least squares means for each attribute were separated using least significant differences (F test, $P < 0.05$).

III. RESULTS AND DISCUSSION

pH decline

AES had no effect on pH decline ($P > 0.05$). AES did not result in an immediate fall in pH as expected (Table 1) when compared to our previous findings [2]. This could be due to the electrical inputs into the carcasses from head-only electrical stunning and immobilization that masked any effect of additional electrical stimulation. Pre-rigor holding temperature (for 3hrs) and time significantly influenced pH fall ($P < 0.001$). pH was lowest at 6hrs *post mortem* except for 35°C samples where the lowest pH was attained at 3hrs *post mortem*. It is well documented

that high *pre-rigor* temperature accelerates *post mortem* pH decline [2, 7].

Table 1 Effect of AES and 3hrs *pre-rigor* holding temperature on rate of pH fall of bull beef LL samples.

	pH fall (average)							
Time *post-mortem*	AES				NES			
	5°C	15°C	25°C	35°C	5°C	15°C	25°C	35°C
before ES	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6
after ES	6.5	6.5	6.5	6.5	6.6	6.6	6.6	6.6
3hrs	6.6	6.4	6.3	6.2	6.6	6.4	6.3	6.2
6hrs	6.4	6.3	6.2	6.1	6.4	6.3	6.2	6.1
24hrs	6.2	6.2	6.2	6.1	6.2	6.1	6.2	6.1
48hrs	6.1	6.2	6.2	6.1	6.1	6.1	6.1	6.1
14 days	6.2	6.2	6.2	6.1	6.2	6.2	6.1	6.1

AES – additional electrical stimulation; NES: non electrical stimulation, n=12; overall SED = 0.07. Treatment effect [AES vs NES] ($P > 0.005$); temperature effect ($P < 0.001$); time effect ($P < 0.001$); Interaction effects ($P < 0.001$).

Table 2 Effect of AES and 3hrs *pre-rigor* holding temperature methods on shear force and sarcomere length of beef LL samples.

Trait	AES				NES			
	5°C	15°C	25°C	35°C	5°C	15°C	25°C	35°C
SF[A]	11	10	10	9	11	10	10	10
SF[B]	6.8	6.4	6.2	6.2	6.4	6.5	6.6	7.0
DL[C]	2.0	1.6	1.4	2.1	2.2	1.6	1.7	2.1
DL[D]	2.0	1.8	1.3	1.7	2.2	2.2	2.1	2.1
PL[E]	3.6	3.3	2.3	3.1	3.1	3.7	3.3	4.0
WHC[F]	5.6	5.1	3.6	4.8	5.3	5.8	5.4	6.1

AES – additional electrical stimulation; NES – non electrical stimulation, n=13. [A]Shear force (48hrs); Treatment effect [AES vs NES] ($P > 0.05$); temperature effect ($P = 0.042$); Interaction effect ($P > 0.05$, SED = 0.62). [B]Shear force (2 weeks). Treatment effect [AES vs NES] ($P > 0.05$); temperature effect ($P > 0.05$); Interaction effect ($P > 0.05$, SED = 0.22). [C]Drip loss (48hrs); Treatment effect [AES vs NES] ($P > 0.05$); temperature effect ($P < 0.001$); Interaction effect ($P > 0.05$, SED = 0.17). [D]Drip loss (2 weeks). Treatment effect [AES vs NES] ($P < 0.015$); temperature effect ($P = 0.005$); Interaction effect ($P < 0.029$, SED = 0.19). [E]Purge loss; Treatment effect [ES vs NES] ($P < 0.001$); temperature effect ($P < 0.001$); Interaction effect ($P = 0.03$, SED = 0.04). [F]WHC (2 weeks); Treatment effect [AES vs NES] ($P > 0.05$); temperature effect ($P < 0.001$); Interaction effect ($P < 0.009$, SED = 0.11).

Shear force

The AES treatment applied to the hot-boned loin samples did not result in significant ($P > 0.05$) changes in shear force values. At 48hrs *post mortem*, shear force values were not significantly different for the AES samples when compared to the NES samples. The 3hrs *pre-rigor* holding temperature had significant ($P = 0.042$) effect on reducing the shear force values, where AES-35 samples had most tender meat when compared to the AES-5 [2, 3]. The trend was not observed in the shear force values at 14 days *post mortem* (Table 2), where there was no significant difference between the shear force values for AES and NES samples due to AES and 3hrs *pre-rigor* holding ($P > 0.05$).

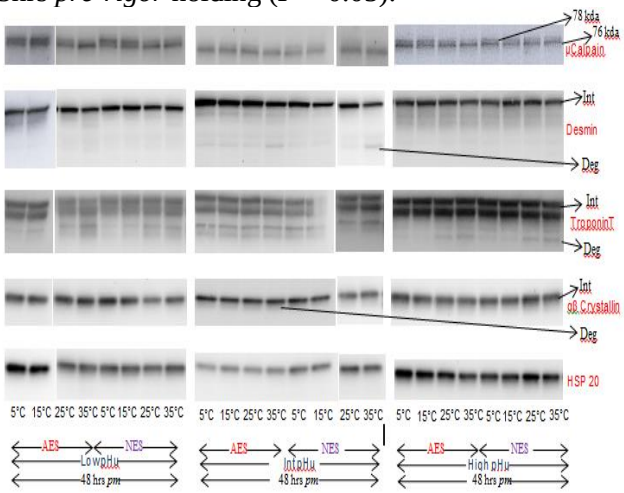

Figure 1 Representative Western blot depicting μ-calpain, desmin, troponin-T, αβ-crystallin, and HSP20 of whole muscle extraction of the beef samples (48hrs *post mortem*) run on 12% gels (except μ calpain run in 7.5% gels). AES – Additional electrical stimulation; Int – Intact protein; Deg – degraded protein; Intr – intermediate pH; NES – non electrical stimulation; *pm* – post mortem.

Water holding capacity

The AES applied to the hot-boned loin samples did not influence drip loss at 48hrs *post mortem* (Table 2; $P > 0.05$). However, the 3hrs *pre-rigor* holding temperature had significant effect on the drip loss. For both AES and NES samples, drip loss was higher for 5°C and 35°C and lower for 15°C and 25°C. After 14 days of ageing, drip loss was significantly influenced by AES, *pre-rigor* holding temperature and their interaction effect ($P = 0.029$). The influence of AES on purge loss was dependent on *pre-rigor* holding temperature (Table 2, $P = 0.03$). Among, the AES samples, AES-25 had significantly lower purge than AES-5. The water

holding capacity (WHC % loss) data presented in this short paper is defined as the sum of the purge and the drip loss values, where lower values denote higher WHC and vice versa. The interaction between AES and *pre-rigor* holding temperature significantly influenced the WHC (Table 2; $P = 0.009$) [8]. A significant increase in WHC was observed for AES-25 when compared to the NES-25 treatment.

Myofibrillar protein and sHSP analysis

The extents of desmin and troponin-T degradation are well-known indicators of meat tenderization [9]. Myofibrillar and sHSP degradation was influenced by AES, 3hrs *pre-rigor* holding temperature and pHu (Figure 1). Generally, AES resulted in less myofibrillar protein (e.g. desmin) degradation compared to NES samples. Among the 3hrs *pre-rigor* holding temperatures, mostly 25°C was found to have higher myofibrillar and sHSP degradation compared to the 5°C samples. Among the three pHu's, desmin degradation was greater in high pHu samples, troponin-T, sHSP20 and αβ-crystallin degradations were higher in intermediate pHu samples, compared to the other pHu categories. Ageing for 14 days resulted in complete µ-calpain autolysis, higher myofibrillar protein (desmin and troponin-T) and sHSP (αβ-crystallin and HSP20) degradation. These findings are in agreement with a recent publication [4] in which a significant correlation between the degradation of sHSP and myofibrillar proteins in beef samples (degraded sHSP27 and desmin) were reported [4, 10].

IV. CONCLUSION

AES had no effect on pH decline, purge and drip loss at 48hrs *post mortem*, but after 14 days of ageing; drip loss was lower due to AES. The 3hrs *pre-rigor* holding temperature did have significant effect on pH decline, shear force values, drip loss and myofibrillar degradation. Overall, this study demonstrate that the *pre-rigor* holding temperature (25°C) alone or in combination with AES resulted in lower shear force values, and increased WHC in bull beef samples.

ACKNOWLEDGEMENTS

This project was supported by the AGMARDT POSTDOC Fund (A19076). The authors would like to thank Greenlea Premier Meats Limited, Hamilton for providing samples and Pete Dobbie, and Kevin Taukiri for their assistance with data collection.

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