PHYSIOLOGICAL UNDERSTANDING OF ELECTRICAL STIMULATION

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Introduction

It is well established in the meat processing industry that electrical stimulation enhances meat tenderness (Devine *et al.*, 2004), generally by hastening the process of rigor mortis. This results in an initial pH fall (ΔpH) followed by a change in the rate of pH decline. Within Australia, a new type of electrical stimulation system has been developed and is currently being implemented in sheep processing plants. These new mid-voltage stimulation (MVS) units are designed to impart the same type of response observed with traditional high voltage stimulation, but without the associated danger and cost (Devine *et al.*, 2004). This is achieved with shorter pulse widths and medium range voltages, delivered through segmented electrodes. Although there have been numerous studies conducted on optimising the effectiveness of stimulation by altering the electrical parameters, such as frequency, pulse width and current (Devine *et al.*, 2004), there have been few detailed studies on MVS units (Pearce *et al.*, 2006). Furthermore, few studies have clarified the physiological interaction and effects of stimulation on muscle contraction, nerve function and pH decline (Chrystall and Devine, 1978; Chrystall *et al.*, 1980; Devine and Chrystall, 1984), hence the effects on meat tenderness remain unclear. In many circumstances, the protocol and physiological effects are defined empirically, based on experience rather than an integrated understanding of the applied electrical parameters and the induced response in the muscle. Given the difficulties associated with studying the subtleties of electrical stimulation on muscle/nerve components in large animals, the use of an isolated nerve-muscle preparation circumvents these problems whilst allowing a detailed study of stimulation characteristics (Devine and Chrystall, 1984). Therefore, the emphasis of this preliminary study was to examine several parameters of MVS systems, particularly frequency and pulse width, and relate them to the mechanical response of muscle (i.e. amount of force generated) and the accompanying biochemical changes (ΔpH).

Materials and Methods

In the abattoir, muscle pH of the *M. semimembranosus* (SM) was measured using an Orion 250A pH meter with a glass body, spear-tipped probe (Orion Research Inc, MA), coupled with a temperature probe. pH and temperature measurements were taken within 1 min prior to stimulation and within 5 min post-stimulation. Table 1 outlines the treatments used for muscle pH. Thirteen consignments were tested, using 5 sheep per treatment (Tmt) and 8 treatments per consignment (Tmt 0 was the unstimulated control). Tmt 1 to 4 had constant current, pulse width and frequency across all 6 electrode bars of the MVS unit. Tmt 5 to 8 had a constant current and pulse width, but different frequencies across the electrodes, e.g. Tmt 5 had a frequency of 15Hz for the first 2 electrodes, 20 Hz for the next 2 electrodes, and 25Hz for the last 2 electrodes. The total duration of stimulation for all Tmts was 34 seconds. For the *in vitro* preparation, rats (male Wistars, aged 10–16 weeks) were deeply anaesthetized with carbon dioxide and decapitated using a guillotine. The SM muscles were immediately removed and immersed in Krebs solution at room temperature. Longitudinal strips (avg 22 x 3mm, 0.132g) of SM muscle were mounted vertically in organ baths containing Krebs solution at 34°C aerated with 5% CO_2 in 95% O_2 (pH 7.4; Chen & Creed, 2004). Following equilibration and before the use of the test parameters, the muscle was stimulated with 80Hz, 0.1ms and 60V to act as a control twitch for each strip, with subsequent responses analysed as a percentage of the control tension generated. Table 2 outlines the parameters used for the in vitro preparations to correlate the ΔpH with tension responses. These parameters were chosen to reflect those used in the abattoir. For muscle pH, data was analysed using an ANOVA and Tukey's Post Hoc test, with Tmt as a fixed variable. Analysis of the nerve/muscle preparation data (the percentage of tension generated, relative to the control twitch) used an ANOVA with treatment as fixed variable.

Results and Discussion

The results presented in Table 1 reveal that modulating the frequency (i.e. altering the frequencies during the stimulation period) produced the greatest influence on the drop in muscle pH, compared with other treatments that had a constant frequency. Furthermore, altering the pulse width had no effect on ΔpH. These results are in agreement with those of Chrystall and Devine (1978), who found that compared to other electrical parameters, pulse frequency had a greater effect on ΔpH and the amount of tension produced. However, results from our preliminary in vitro study (Table 2) found that modulating frequencies had no effect on tension generated, whereas varying pulse width did.

Table 1: Effect of electrical stimulation on muscle pH (mean ± S.E.).

Tmt No.	Frequency (Hz)	Pulse width (ms)	Current (A)	No. of Sheep	Initial pH	Post-stim pH
0		No stimulation		67	6.89 ± 0.03	6.77[a] ± 0.04
1	10	1	0.75	67	6.87 ± 0.04	6.54[b] ± 0.06
2	40	1	0.75	67	6.81 ± 0.04	6.52[b] ± 0.04
3	15	1	0.75	67	6.86 ± 0.04	6.47[ab] ± 0.03
4	15	5	0.75	67	6.86 ± 0.04	6.49[ab] ± 0.04
5	15, 15, 20, 20, 25, 25	1	0.75	67	6.91 ± 0.05	6.46[c] ± 0.03
6	25, 25, 20, 20, 15, 15	1	0.75	67	6.89 ± 0.03	6.77[a] ± 0.04
7	25, 25, 20, 20, 15, 15	3	0.75	67	6.87 ± 0.04	6.54[b] ± 0.06
8	25, 25, 20, 20, 15, 15	5	0.75	67	6.81 ± 0.04	6.52[b] ± 0.04
	Ave				6.86 ± 0.24	6.59 ± 0.28

* Treatments followed by different superscripts are significantly different ($P<0.05$)

Table 2: Effect of electrical stimulation on muscle tension.

Treatment	%tension
15hz 1ms 60v	23.58
15hz 5ms 60v	47.32
15hz 2.5ms 60v	40.98
15hz 1ms 100v	38.63
15hz 1ms 30v	17.85
i. 15hz 1ms 60v	19.93
ii. 20hz 1ms 60v	21.93
iii. 25hz 1ms 60v	25.53

Conclusion

We believed that a reasonable assumption would be that the biggest drop in muscle pH stems from the greatest peak contraction. However, this appears not to be the case. The magnitude of ΔpH is governed not only by stimulation characteristics (e.g. frequency and pulse width) but also muscle temperature and post-mortem delay of stimulation (Devine *et al.*, 2004). Further studies are required to investigate the influence that each of these elements have on ΔpH and the corresponding tension produced.

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