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INTER-MUSCULAR DIFFERENCES IN RESPONSE TO LOW VOLTAGE ELECTRICAL STIMULATION OF LAMB

NFS Gault*[1,2], A Gordon[3] and ELC Tolland[2]

Department of Food Science[1], Queen's University Belfast; Agriculture, Food and Environmental Science Division[2], and Corporate Services Division[3], Department of Agriculture for Northern Ireland, Belfast BT9 5PX, UK

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Introduction

Traditional electrical stimulation (ES) increases the rate of post mortem glycolysis in red meat carcasses, hence facilitating rapid chilling pre-rigor and avoiding cold shortening (Chrystall and Devine 2000). However, extensive ES may inadvertently induce heat shortening, thus adversely affecting eating quality. Hence, most conventional ES procedures aim to decrease the pH of the *longissimus dorsi* muscle to no less than pH 6.3 1h post mortem. Conversely, Gault *et al.* (2000) demonstrated that low voltage electrical stimulation (LVES) of chicken carcasses could induce a rapid drop in the breast muscle to pH 6.0, inducing very early rigor onset, yet having no detrimental effect on eating quality. This was achieved using only 300 pulses of 100v LVES applied at various duty cycles (Li *et al*. 1993) to induce different rates of wing flapping, as opposed to the tetanus inducing duty cycles used in conventional red meat ES systems.

Unlike chicken breast muscle which comprises mainly αW (fast-twitch high glycolytic) muscle fibres (Sams and Janky 1990), the muscles of red meat animals consist of varying proportions of αW, αR (fast-twitch high oxidative) and βR (slow-twitch intermediate/high oxidative) muscle fibre types (Monin 1981). Thus their responsiveness to carcass LVES should reflect how the electrical parameters used affect the physiological processes that invoke muscle contraction and anaerobic energy utilization.

Objectives

To assess (a) the effectiveness of the glycolytic response of different muscles in lamb carcasses to low frequency LVES treatments which induce separate muscle contractions, thus avoiding complete tetanus, and (b) the effect of these treatments on the quality characteristics of muscles de-boned early and at 24h.

Methodology

Animals and treatments

This study used 40 (24 male; 16 female) Texel x Greyface crossbred lambs; mean live weight 44.76 kg (range 35-56kg). Groups of 4 lambs were randomly allocated to one of 9 treatments in which 300 pulses of LVES, at a constant 100v, were applied to each carcass 90s after captive bolt stunning and bleeding. The 3 x 3 + 1 split plot factorial design comprised the use of frequencies of 1, 3, and 5 Hz at pulse widths of 10, 50 and 100 ms. These equate to duty cycles of 1%, 3%, 5%, 10%, 15%, 25%, 30% and 50%. A control group of 4 lambs received no LVES treatment. All carcasses were dressed and split within 10 minutes of bleeding.

Muscles & de-boning

The muscles selected for assessment were the *semitendinosus* (ST); *longissimus dorsi* (LD); *vastus lateralis* (VL); *semimembranosus* (SM); *gluteus medius* (GM); *triceps brachii* (TB); *supraspinatus* (SS) and *infraspinatus* (IS). These represent a broad range of muscle types differentiated by their varying proportions of αW, αR and βR muscle fibre types respectively (Monin, 1981).

Muscles from alternate carcass sides within each treatment were removed for analysis immediately after splitting (early de-boned), while the matching sides were Achilles hung until 24h post-mortem (late de-boned). Chilling was carried out at 9^{o}C for the first 24h post-slaughter. Muscles were then stored aerobically for one week at 2^{o}C when the remaining analyses were completed.

Meat quality measurements

Muscle pH decline was monitored at 1h, 2h and 24h post mortem in each of the early de-boned muscles, and at 24h in the late de-boned muscles. Approximately 1g samples of muscle were frozen in liquid nitrogen until analyzed by the iodoacetate method of Bendall (1975). Sarcomere lengths were measured in samples of early and late de-boned muscles at 48h post-mortem by the method of Koolmees *et al* (1986), and cooking loss and shear force assessed (Gault *et al*, 2000) at 48h and 7 days post mortem. Data were analyzed by ANOVA (Genstat, 2003).

Results & Discussion

Intermuscular and treatment effects on quality parameters - whole carcasses

The primary findings of this experiment were in identifying significant ($P<0.001$) intermuscular differences amongst all of the quality traits measured for all treatments combined (Table 1), namely pH after 1h, 2h and 24h; and sarcomere length, cooking loss and shear force values after 48h and 7 days storage. This simply confirms the wide range

in metabolic activity, chemical composition and impact of anatomical location of the muscles chosen.

Likewise, a comparison of the quality traits measured between the control treatment and those of the combined LVES treatments for all muscles combined (Table 2) shows that the LVES treatments gave significantly lower ($P<0.001$) mean pH values at 1h and 2h post-slaughter. In contrast, LVES, taken as a combined treatment, had no significant effect ($p>0.05$) on the other quality parameters compared to the control, the only exception being the greater cooking losses at 48h ($P<0.01$) due to LVES.

Pulse width and frequency of LVES were also analyzed as independent treatments against the control treatment for all muscles combined. In general, neither frequency nor pulse width had a significant effect ($P>0.05$; data not shown) on any quality parameter. The only significant interaction found was that between pulse width and cooking loss at 48h ($P<0.01$; data not shown), similar to that found between the combined LVES treatments and the control in Table 2. Consequently, although the combined LVES treatment significantly increased the glycolytic response of all muscles (Table 2), the combinations of pulse width and frequency of LVES chosen had little specific influence on any quality parameter. This strongly suggests that the combinations of pulse width and frequency used were equally effective in enhancing a glycolytic response without adversely affecting other quality characteristics.

The effects of control and combined LVES treatments on the pH values of individual muscle groups are shown in Table 3. In the control carcasses, the order of mean pH fall 1h after slaughter followed the expected trend in glycolytic response related to predominant muscle fibre type in lamb muscles (Monin, 1981). The predominantly αW fibre-type ST gave the greatest decrease, the αR LD and SM an intermediate response, and the βR SS and IS the least response. Likewise, for the ES carcasses, a similar trend was observed, the main exception being the unexpectedly large pH fall in the TB, a predominantly βR metabolic type muscle. The low pH_{1h} values of the ST and TB suggest that these muscles were very close to entering rigor. In contrast, when using pH decline as a measure of glycolytic responsiveness to LVES, the other muscles were much less responsive, especially the SS, VL and LD. Intermuscular pH differences at 2h reflected those at 1h, whereas those at 24h reflected normal intermuscular biochemical differences attributable to glycogen reserves and fibre type distribution (Table 3).

In contrast to the intermuscular treatment interactions shown in Table 3, no significant interactions ($P>0.05$; data not shown) were found for any of the other quality parameters measured.

Pulse width and frequency of LVES were also analyzed as independent treatments against control values in relation to individual muscle groups (data not shown). The only significant interactions found were those between pulse width and pH_{1h} ($P<0.05$), pulse width and cooking loss at 48h ($P<0.01$), and pulse width and shear force value at 48h ($P<0.05$). Frequency of LVES gave no significant interaction ($P>0.05$) between any of the meat quality parameters measured and individual muscle groups.

Intermuscular and treatment effects on quality parameters - early and late de-boning

Early de-boning for all treatments and muscles combined (Table 4) resulted in significantly shorter sarcomeres and higher shear force values at both 48h and 7d (all $P<0.001$). There was no significant effect of de-boning time on cooking losses.

Likewise, early de-boning for all treatments combined brought about similar intermuscular effects (Table 5), sarcomeres being significantly shorter ($P<0.001$) and shear forces at both 48h and 7d significantly higher ($P<0.001$) than the comparable muscles de-boned after 24h. There were no significant intermuscular effects or interactions on cooking losses ($P>0.05$; data not shown).

Interestingly, a significant combined muscle x LVES x control treatment interaction ($P<0.05$) was found for shear force values at 48h (Table 6). Although early de-boning clearly induced an increase in shear force, this was generally significantly less for the LVES treatments. As expected, there was generally no difference in shear force values between the control and LVES treatments for the 24h de-boned muscles. The effect of the interactions between pulse width and frequency of LVES are difficult to interpret, although there would appear to be a trend suggesting that the lowest shear forces may be found with higher frequency x pulse width combinations, i.e. at higher duty cycles.

Conclusions

This study has confirmed that important intermuscular differences exist in the glycolytic response of lamb carcasses to pulsed LVES treatments. The greatest response was seen with predominantly αW fibre-type muscles, and the least with predominantly βR fibre-types, some muscles reaching pH 6.0 within 1h of slaughter. While significant intermuscular differences in all other quality parameters were found, the LVES treatments used had no significant effect on these. In contrast, early de-boning resulted in significantly higher shear force values after 48h for all muscles combined. However, these were significantly lower for those from the LVES treated carcasses, even though some of these muscles would have entered rigor early. This merits further study on the use of extended LVES to minimize the adverse effects of early de-boning of lamb carcasses.

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Tables and Figures

Table 1: Intermuscular differences in the quality attributes of lamb.

Muscle	pH_{1h}	pH_{2h}	pH_{24h}	SL_{48h} µm	CL_{48h} %	CL_{7d} %	SF_{48h} $kgcm^{-2}$	SF_{7d} $kgcm^{-2}$
ST	6.10	6.01	5.80	2.11	33.0	33.5	7.51	5.02
LD	6.37	6.29	5.60	1.87	25.4	27.0	5.44	4.05
SM	6.32	6.20	5.68	1.91	30.0	31.7	6.11	4.58
VL	6.41	6.30	5.80	1.93	30.3	31.0	6.11	3.91
GM	6.35	6.27	5.66	1.86	27.3	29.0	5.80	4.15
TB	6.22	6.13	5.79	2.15	26.3	27.6	5.34	3.45
SS	6.56	6.41	5.91	1.86	32.5	32.3	5.46	3.94
IS	6.42	6.31	5.90	1.84	25.3	24.4	5.09	3.56
mean	6.34	6.24	5.77	1.94	28.8	29.6	5.86	4.08
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
n	40	40	80	80	80	80	80	80
l.s.d. (P<0.05)	0.05	0.05	0.02	0.06	0.8	0.7	0.32	0.21

Table 2: Effect of combined LVES v control treatments on the quality attributes of lamb for all muscles combined.

Treatment	pH_{1h}	pH_{2h}	pH_{24h}	SL_{48h} µm	CL_{48h} %	CL_{7d} %	SF_{48h} $kgcm^{-2}$	SF_{7d} $kgcm^{-2}$
Control	6.59	6.48	5.73	1.92	26.62	28.29	6.06	4.41
LVES	6.32	6.21	5.77	1.94	28.99	29.70	5.84	4.04
P	<0.001	<0.001	NS	NS	<0.01	NS	NS	NS
n	32/288	32/288	64/576	64/576	64/576	64/576	64/576	64/576
l.s.d. (P<0.05)	0.11	0.10	0.09	0.07	1.51	1.96	0.76	0.52

Table 3: Effect of combined LVES treatments on inter-muscular pH values compared to control values.

Muscle	pH_{1h} control	pH_{1h} LVES	pH_{2h} control	pH_{2h} LVES	pH_{24h} control	pH_{24h} LVES
ST	6.46	6.06	6.38	5.97	5.85	5.80
LD	6.54	6.35	6.42	6.28	5.56	5.60
SM	6.55	6.29	6.41	6.18	5.61	5.68
VL	6.58	6.39	6.43	6.29	5.80	5.80
GM	6.59	6.32	6.45	6.26	5.62	5.67
TB	6.61	6.18	6.53	6.08	5.71	5.80
SS	6.66	6.54	6.63	6.38	5.87	5.92
IS	6.72	6.39	6.62	6.27	5.56	5.60
mean	6.59	6.32	6.48	6.21	5.73	5.77
P	<0.01		<0.001		<0.01	
n	4	36	4	36	8	72
l.s.d.[a]	0.16	0.05	0.15	0.05	0.07	0.02
l.s.d.[b]	0.16		0.15		0.10	

[a] least significant difference (P<0.05) for means within a column.
[b] least significant difference (P<0.05) for means across each pair of columns.

Table 4: Effect of de-boning time on quality attributes of all muscles combined.

De-boning time	pH_{1h}	pH_{2h}	pH_{24h}	SL_{48h} µm	CL_{48h} %	CL_{7d} %	SF_{48h} $kgcm^{-2}$	SF_{7d} $kgcm^{-2}$
early	-	-	5.76	1.81	29.13	29.54	6.46	4.51
24h	-	-	5.77	2.07	28.37	29.56	5.26	3.66
P	-	-	<0.05	<0.001	NS	NS	<0.001	<0.001
n	-	-	320	320	320	320	320	320
l.s.d. (P<0.05)	-	-	0.01	0.06	0.81	0.68	0.14	0.11

Table 5: Effect of de-boning time on quality attributes of individual muscles.

Muscle	pH_{24h} early	pH_{24h} 24h	SL_{48h} early	SL_{48h} 24h	SF_{48h} early	SF_{48h} 24h	SF_{7d} early	SF_{7d} 24h
ST	5.79	5.81	1.92	2.31	8.36	6.66	5.79	4.25
LD	5.61	5.58	1.82	1.91	5.97	4.92	4.38	3.72
SM	5.68	5.67	1.87	1.94	6.25	5.96	4.62	4.55
VL	5.81	5.80	1.85	2.02	6.93	5.29	4.37	3.44
GM	5.67	5.67	1.81	1.89	6.29	5.32	4.41	3.89
TB	5.79	5.79	1.81	2.49	5.99	4.70	3.92	2.98
SS	5.89	5.94	1.73	1.98	5.95	4.96	4.29	3.59
IS	5.85	5.94	1.70	1.99	5.94	4.24	4.28	2.83
mean	5.76	5.77	1.81	2.07	6.46	5.26	4.51	3.66
P	<0.001		<0.001		<0.001		<0.001	
n	40	40	40	40	40	40	40	40
l.s.d.[a]	0.03	0.03	0.08	0.08	0.45	0.45	0.30	0.30
l.s.d.[b]	0.03		0.08		0.44		0.30	

[a] least significant difference (P<0.05) for means within a column.
[b] least significant difference (P<0.05) for means across each pair of columns.

Table 6: Effect of de-boning time on combined muscle shear force values at 48h as a function of individual LVES and control treatments.

De-boned	PW(ms)			Frequency (Hz)			mean (PW)
				1Hz	3Hz	5Hz	
early[a]		control	**6.90**				
	10ms			7.12	7.17	6.07	6.79
	50ms			6.24	5.85	6.48	6.19
	100ms			6.13	6.55	6.10	6.26
	mean (F)			6.50	6.52	6.22	**6.41**
24h[a]		control	**5.22**				
	10ms			5.62	5.50	5.29	5.47
	50ms			5.46	5.20	5.24	5.30
	100ms			4.85	5.27	4.92	5.01
	mean (F)			5.31	5.32	5.15	**5.26**
P			<0.01	<0.01	<0.01	<0.01	
reps			32	32	32	32	
l.s.d.[b]			1.06	1.06	1.06	1.06	

[a] least significant difference (P<0.05) = 0.44 for all means within each de-boning period.
[b] least significant difference (P<0.05) for all treatment means.