INTERACTION OF PRE-SLAUGHTER STRESS AND LOW VOLTAGE ELECTRICAL STIMULATION ON MUSCLE PROTEOLYTIC ENZYMES AND MEAT TENDERNESS OF LAMBS

R Bickerstaffe[1], J D Morton[1], C C Daly[2], and G M Keeley[3]

[1]Lincoln University, Canterbury, New Zealand; [2]Meat Industry Research Institute, Hamilton, New Zealand; [3]Canterbury Frozen Meat, Christchurch, New Zealand

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Introduction

Deterioration in meat quality following pre-slaughter stress can, in many cases, be attributed directly to the elevation in ultimate pH. For example, a increase in ultimate pH has been associated with a reduction in the storage time of chilled meat, darkening of meat colour, decreased drip loss and changes in tenderness (Guignot *et al.*, 1994, Apple *et al.*, 1995). The effect of ultimate pH on tenderness is, however, complex with evidence sugge that meat with an ultimate pH <5.8 should be tender, 5.8-6.2 tough and >6.2 tender (Purchas and Aungsupakorn, 1993, Watanabe *et al*, 1996). Hi voltage electrical stimulation (HVES) has been used to accelerate the onset of rigor mortis and, consequently, reduce the time for muscles to reach t ultimate pH (Shorthose *et al.*, 1986). Although HVES is not a tenderising process, improvements in tenderness and other meat quality characteristi have been associated with the process. Nevertheless, despite the use of HVES and ideal chilling regimes to attain ultimate pH values <5.8, unaccept tough meat can be produced. Such observations indicate there is a meat toughening factor which is independent of ultimate pH and HVES.

Objectives

This paper investigates possible contributors to an ultimate pH independent toughening factor by quantifying the effect of various levels of pre-slaug stress and the interaction of stress with low voltage electrical stimulation (LVES) on creatine kinase, muscle proteolytic enzymes and tenderness.

Methods

A mob (n=205) of 8 month old stress tolerant Borderdale x Suffolk lambs were swim washed, except for 5 (controls), on arrival at a processing plan and divided into two groups, A and B. Group A lambs (n=100) were minimally stressed by holding the animals in a quiet environment next to the processing access ramp without any dog handling and processed 16h post swim wash. After stunning the carcasses were randomly processed with (n=80) or without (n=20) LVES (90 Vpk 30 sec). All carcasses were subsequently subjected to HVES (1130 Vpk 90 sec) within 25 min post-stunni Group B lambs (n=100) were moderately stressed by holding the lambs in the centre of the stock yards overnight, swim washing a second time 3h pr to slaughter and assembling the animals to the access ramp using dogs. The lambs were processed 3h later than Group A using identical processing conditions. Carcasses were processed with (n=80) or without (n=20) LVES. The pH and temperature of the *longissimus dorsi* (LD) muscle was measured immediately before (25 min post-stunning) and at intervals after HVES using an Orion 8163 spear pH electrode attached to a Hanna 9025 meter over the first 27h post-mortem and, subsequently, on vacuum packed meat removed for tenderness determinations. Samples (5g) were remove from the LD from six animals of each treatment group at 25 min, 12h and 27h post-stunning and homogenised immediately in six volumes of homogenisation buffer (100 mM Tris-HCl, 10mM merceptoethanol, 10mM EDTA, 100 mg/L ovomucoid, 2.5 µM E64, 2mM PMSF, pH 8.3) using Ultra Turrax. Proteases were separated on DEAE-sepharose FF and assayed as described by Morton & Bickerstaffe (1996). At 4h, 12h, 27h, 7d an 14d post-stunning, 45mm LD muscle steaks were assessed for tenderness using a MIRINZ tenderometer (Devine and Graafhuss, 1995).

Carcasses were chilled at 15°C for 8h, 10°C for 4h and 1°C for 15h. After 27h, the carcasses were boned out, LD muscle vacuum packed and held at -1°C for up to 2 weeks. Creatine kinase activity was determined in blood 30 sec post LVES and within 2 min post-stunning.

Results

Moderate stress to animals had no significant effect on the ultimate pH of the LD but there was an effect on the rate of pH fall 75 min post-stunning i the LVES carcasses (Table 1). In the carcasses without LVES, there was a tendency for meat to be tougher from the moderately stressed lambs (Fig. 1). Application of LVES to carcasses from moderately stressed animals had no effect on meat toughness. In contrast, LVES of carcasses from low stressed animals significantly increased ($p < 0.05$) the toughness of meat (Fig. 1). These differences in toughness were evident in the LD muscles to 14 days post-mortem (Fig. 1). There was no significant differences in ultimate pH values in any of the treatments; all were <5.8 (Table 1). Thus, meat toughening factor independent of ultimate pH is evident when LVES is applied to minimally stressed animals. Examination of the proteolytic enzymes showed there was no significant differences in µ-calpain, m-calpain and calpastatin activities between LVES and non-LVES muscles from moderately stressed animals or non-LVES muscles from minimally stressed animals. Application of LVES to minimally stressed animals, however, significantly reduced µ-calpain captivity 25 min post-stunning (Table 1). Associated with this difference in muscle proteolytic potential were significa increases in meat toughness although ultimate pH was <5.8. Plasma creatine kinase was significantly lower in animals receiving LVES.

cussion

ere is considerable evidence that exposing animals to pre-slaughter stress can increase ultimate pH to 5.8-6.2 and associated with this an increase in at toughness (Purchas and Aungsupakorn, 1993). Changes in tenderness have also been linked with rapid rates of pH fall (Marsh *et* al., 1987; mulders *et al.*, 1990). However, there is no evidence that pH is directly responsible for the increase in toughness. Indeed, changes in meat tenderness n occur independently of pH or rate of pH change. For example, pH independent tenderness changes have been linked to (1) interaction between pre-ughter animal stress with LVES of carcasses (Daly *et al.*, 1995) or (2) activation of calcium dependent proteases by calcium (Koohmaraie *et al.*, 90). The results in this paper confirm that combining minimal levels of pre-slaughter stress with low voltage electrical stimulation of carcasses reases meat toughness compared to exposing animals to moderate stress. Examination of muscle proteolytic enzymes showed that the combination of inimally stressed animals and low voltage electrical stimulation of the carcasses decreased muscle μ-calpain activities 25 min post-stunning. The results ggest that the reduction in μ-calpain activity is the primary contributor to the pH independent meat toughening process. The exact role of any changes Ca^{++} flux or the modulating effect of calpastatin on μ-calpain under these experimental conditions have yet to be determined.

nclusions

nificant increases in meat toughness occurred in animals subjected to minimal levels of pre-slaughter stress and muscles to low voltage electrical mulation. The associated reductions in muscle μ-calpain activity indicates that muscle proteolytic enzymes are a major factor contributing to the pH pendent toughening process.

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Table 1. Effect of stress and low voltage electrical stimulation (LVES) on plasma creatine kinase and *Longissimus Dorsi* pH and proteases in lamb carcasses. All times are post-slaughter. Numbers with different superscripts are significantly different.

Stress	LVES	Creatine kinase	pH 75 min	pH Ultimate	μ-calpain 25 min	μ-calpain 12 h	m-calpain 25 min	m-calpain 12 h	calpastatin 25 min	calpastatin 12 h
None	Yes	160[a]	5.91	5.69						
Minimal	No	302 [b]	5.98	5.71	1.39[a]	0.49	1.74	1.70	4.32	1.84
	Yes	188 [a]	5.89	5.76	1.06[b]	0.5	1.81	1.81	3.33	2.32
Moderate	No	296[b]	5.96	5.67	1.54	0.63	1.54	1.96	3.64	2.04
	Yes	162 [a]	6.12	5.73	1.62	0.71	1.73	1.88	3.75	1.83

Figure 1. The effect of stress and low voltage electrical stimulation on tenderness in lamb carcasses.