Effect of Pre-Slaughter Stress on Beef Quality

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Introduction

The way an animal is treated prior to slaughter can alter the quality of the meat produced from that animal. One of the mechanisms is that pre-slaughter stress reduces the glycogen content of the muscle. These muscles produce less lactic acid post mortem and, consequently, result in meat of high ultimate pH. This meat has a dark colour, reduced flavour and is susceptible to bacterial spoilage (Lister *et al*, 1981).

The effect of ultimate pH on the tenderness of meat is more complex. There is evidence that tenderness decreases with increasing pH up until ultimate pH's of 6.0-6.2 and then meat of higher pH is more tender (Purchas, 1990; Watanabe *et al*, 1996).

This report considers the effect of spray washing cattle on the quality of the resulting meat. Spray washing is a hygiene requirement to reduce the contamination of carcasses but leads to a visible level of stress to the animals. Our hypothesis is that this stress will lead to meat with higher ultimate pH and altered meat quality characteristics.

Objectives

- To determine the effects of spray washing on some meat quality attributes
- To determine whether the toughness of meat of intermediate pH is a result of a shortening of the myofibrils or an effect on proteolysis (limited ageing) of the myofibrillar proteins.

Methods

A herd of cattle (Devon X) was split into two groups. The first group of 10 was held in pens without any belly wash treatment (dry pens) and the second group of 11 was held in a pen where belly sprays were turned on every hour for five minutes. Animals from the dry pens were killed first whilst the spray-washed animals were killed later in the day.

The following procedures were performed on each longissimus dorsi (LD) samples from the two groups of carcasses: measurement of ultimate pH, shear force determination at 24 hours post mortem, myofibril isolation and myofibril fragmentation index (MFI) determination at 24 hours and 7 days, sarcomere length measurement using phase contrast microscopy and Western blotting of μ-calpain, troponin T and desmin (Geesink *et al*, 2001).

Results and Discussion

The LD muscles from the spray-washed and dry animals were analysed for their ultimate pH and shear force at 24 hours. Spray washing resulted in considerably more variation in the pH of the meat (5.52 to 6.48) compared to meat from the dry animals (5.50 to 5.79). The increased variability in pH was reflected in the meat shear force values from the spray-washed animals which ranged from 5.2 to 14.5 kgF while that from the dry animals ranged between 4.8 and 8.6. The mean pH and the shear force of the meat from the spray-washed animals were higher but the difference was not significant ($p=0.09$, Table 1). This is probably due to the high variability of the measured meat parameters from the spray-washed animals.

The LD samples were further analysed to determine the cause of any toughening. Two factors which can contribute to meat toughness are the state of contraction of the muscle (measured as sarcomere length) and the extent of any post-mortem proteolysis (measured as myofibrillar fragmentation index). The LD's from spray washed animals had shorter sarcomeres than those from dry animals ($p = 0.069$). This is one possible reason for the slightly increased meat toughness associated with the spray washed animals. However the myofibrillar fragmentation index measured at one ($p=0.67$) and seven days ($p=0.40$) post mortem were essentially the same for both spray washed and non-sprayed animals. Thus, there was no evidence that spray washing contributed to the toughening of LD by reducing protein breakdown.

There have been many reports of a relationship between ultimate pH and shear force. In this study the pH was positively correlated with the shear force at 24 hours of ageing ($r = 0.760$). However as shown in Fig.1 the relationship was not linear. The shear force increased as the ultimate pH progressed from 5.6-5.9 and peaked between pH 5.9 and 6.2. As the pH increased above 6.2 the shear force tended to decrease. The results fitted a curvilinear relationship as found in earlier research (Purchas, 1990).

The results of this experiment indicate that ultimate pH is inversely related to the sarcomere lengths ($r = -0.525$). The shortest sarcomere length (1.49 μm) was at an ultimate pH of 5.79. The sarcomere length decreased linearly as the ultimate pH moved towards an intermediate pH range (5.8-6.2). Thereafter, the sarcomere length increased as the ultimate pH increased. This curve is an inverse of that for pH which suggests that the shorter sarcomere lengths may be a cause of the increased shear force (Fig.1).

The ultimate pH could also alter the shear force by its effect on the activity of proteolytic enzymes. The result of proteolysis can be measured by myofibrillar fragmentation index (MFI). After one day of ageing, there was no evidence of a relationship between MFI and ultimate pH although it appears that low ultimate pH (5.47) or high ultimate pH (6.48) meat samples have high MFI values.

Post-mortem proteolysis is generally considered to be due to the action of μ-calpain (Koohmaraie, 1996; Geesink *et al*, 2000). Once μ-calpain is activated it not only degrades other proteins but also it degrades itself, a process named autolysis. Thus it is possible to

measure the activity of the calpains by determining the extent of μ-calpain autolysis. Western blotting of μ-calpain showed that the extent of autolysis at 24 hours was not related to the MFI at day 1 but was related to the MFI at day 7. There was no evidence of an effect of spray washing on μ-calpain autolysis.
Western blotting of two muscle proteins that are substrates for calpain revealed a relationship with tenderness. Troponin T degradation was negatively correlated with pH and shear force. That is, the tough carcasses were those with the least degraded troponin T. However the spray washing had no effect on degradation of troponin T. Desmin was extensively degraded in the seven days post-mortem. The extent of this degradation was related to the MFI and there was a trend (p= 0.079) towards less degradation in the meat from those animals which had been spray washed.

Conclusions

- The limited size of the trial and variability of the meat from spray washed animals prevented any significant results being obtained. However there was a trend towards spray washing causing a rise in ultimate pH and a toughening of the meat. This is more likely to be related to shorter sarcomeres than reduced proteolysis.
- The shear force increased as the ultimate pH reached 6.2 and then decreased.
- Sarcomere length decreased until pH 6.2-6.3 and then increased in a mirror image of shear force.
- Proteolysis, as measured by MFI and Western blotting, increased with pH. Note, however, that although the mean MFI values at pH 5.5 to 5.7 were low, the highest MFI values were from this group.

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Table 1. Effect of spray washing on LD ultimate pH, shear force at 24 hours, MFI at 24 hours, sarcomere length, autolysis of μ-calpain and degradation of tropinin-T and desmin. Autolysis of μ-calpain was determined by the ratio of the density of the higher molecular weight band for the large subunit to the density of the lower molecular weight band. Degradation of tropinin-T and desmin was determined by the relative density of the bands at day 7 and day 1. The values shown are means followed by the standard error of the mean.

	Number of samples	pH	Shear force (kgF)	MFI (1 day)	Sarcomere length (μm)	μ-calpain	Tropinin-T	Desmin
Control	10	5.58 (0.03)	6.3 (0.37)	68 (4.9)	1.86 (0.04)	1.81 (0.13)	0.74 (0.04)	0.03(0.02)
Washed	11	5.77 (0.12)	8.1 (0.89)	71 (2.7)	1.73 (0.05)	2.42 (0.63)	0.76 (0.10)	0.17(0.07)
t-test (p)		0.14	0.09	0.67	0.07	0.37	0.88	0.079

Figure 1. The relationship of LD ultimate pH with shear force at 24 hours post mortem (Δ) and sarcomere length (■).