INVESTIGATIONS INTO WHY BEEF OF INTERMEDIATE ULTIMATE pH IS OFTEN LESS TENDER

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INTRODUCTION

The tenderness of beef longissimus muscle as measured by shear force values has been shown in several studies to decrease as ultimate pH (pH_{ult}) increases from about 5.5 to 6.0, and then to improve with further increases in pH_{ult} to about 7.0 (Bouton et al. 1957; Purchas 1990; Jeremiah et al. 1991). Proposed explanations for the higher shear force values at intermediate relative to low pH_{ult} include a greater myofibrillar shortening (Purchas 1990; Olsson et al. 1995), and a lower level of proteolytic activity (Yu & Lee 1986). The former suggestion is supported by evidence of shorter sarcomeres and by the absence of any effect when muscles are stretched; and the latter by microscopic evidence of less myofibrillar disruption and by slower rates of ageing in some studies (Watanabe et al. 1996), but not in others (Wulf et al. 1996). The aim of the current study was to assess the relationship between myofibrillar breakdown and shear force in samples of beef longissimus muscle with low and intermediate levels of pH_{ult}.

MATERIALS AND METHODS

Samples of longissimus muscle in the 8-12 rib region were taken within 90min *post mortem* from the 156 cattle described by Purchas et al. (1997). These included bulls and steers of several breeds and crosses. From the 156 samples, 24 were selected which included 12 at intermediate pH_{ult} and high Warner-Bratzler (WB) peak shear force, and 12 at low pH_{ult} and low peak shear force. Samples were balanced for bulls and steers and by year. The aim in selecting samples on the basis of both pH_{ult} as well as WB peak force was to have the means for the two groups close to the points for pH_{ult} values of 5.5 and 6.0 on the overall regression curve of WB peak force versus pH_{ult}.

Following ageing at 0-2°C for either 1 or 20 days, muscle samples were frozen at -20°C until processed. Measurements of pH_{ult} (in a homogenate), WB peak force (after cooking at 70°C for 90min), and sarcomere length (by laser diffraction) were made as described by Purchas & Aungsupakorn (1993). Myofibrillar fragmentation indexes (MFI) were measured using a slight modification of the method of Johnson et al. (1990) that involved passing an homogenate through a 231μm screen. Analysis of myofibrillar proteins by SDS-PAGE was conducted according to the method of Claeys et al. (1995), with BSA included as an internal standard at a concentration of 1μg BSA/20μg myofibrillar protein. Myofibrillar extracts (4μg protein/μl) of 5μl and 2.5μl were run for each sample on 7.8% gels with a 4.3% stacker gel. Slabs were stained after electrophoresis with Coomarsie blue and bands were quantified by densitometry. Statistical analysis was by paired t-test for assessing ageing effects, and by oneway analysis of variance for comparing low versus intermediate pH groups.

RESULTS AND DISCUSSION

Figure 1 shows the position of the 24 samples used in this study relative to the polynomial regression line showing the relationship between WB peak shear force and pH_{ult} for the whole population of 156 samples from which the 24 were selected. The overall pattern is similar to that reported previously (eg. Purchas 1990) with the peak in this case at a pH_{ult} of just below 6.0.

Characteristics of the low and intermediate pH groups (Table 1) were as intended with respect to pH_{ult} and WB peak force, but the scatter for the intermediate group was wider because there were fewer samples to select from in that region. The decrease in peak force from 1 to 20 days was greater for the Inter-pH group.

Table 1: Characteristics of the low and intermediate (Inter) pH_{ult} groups.

	Low-pH	Inter-pH	S[a]
Number of samples	12	12	
pH_{ult}	5.55	5.96	0.15***
WB peak shear force (kg)			
1-day	13.01	23.43	3.76***
20-day	10.05	16.03	2.85***
1d - 20d	2.96	7.40	3.10**
MFI(%)[b]			
1-day	81.7	82.3	2.0 ns
20-day	84.0	87.4	3.2 *
1d - 20d	-2.3	-5.1	3.1*
Sarcomere length (μm)	1.52	1.46	0.17 ns

[a] Pooled within-group standard deviation; ns = P > 0.05;* = P < 0.05; ** = P < 0.01 *** = P < 0.001.

[b] Myofibrillar fragmentation index (higher values indicate greater fragmentation).

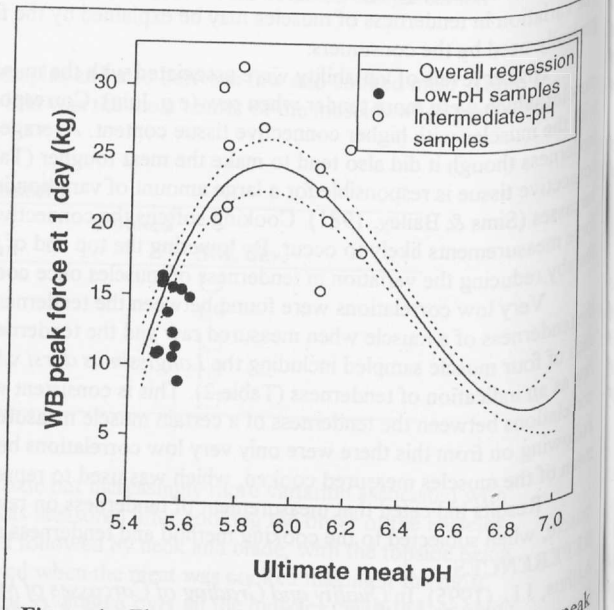

Figure 1: The overall cubic-fit regression line between peak force and pH_{ult} for all 156 samples with 95% confidence intervals (R^2=61%), together with the 24 samples of this study.

MFI values did not differ between the two groups at 1d, but were greater for the Inter-pH group after 20d ageing and the increase from 1 to 20 days was greater for that group. Sarcomere lengths were not significantly shorter for the Inter-pH group as they were for Purchas (1990).

Differences between the Low-pH and Inter-pH groups in the concentration of nine myofibrillar proteins as assessed by SDS-PGE are shown in Table 2 at 1-day of ageing and as the change from 1 to 20 days of ageing. At 1 day the Inter-pH group had significantly lower AA, ACT and

KD30, while the change from 1 to 20 days for the Inter-pH group was less for MHC and greater for ACT. The fact that KD30 was lower for the less tender Inter-pH samples is consistent with the fact that it is usually at lower levels in tougher unaged meat. The size of the effects in Table 2, however, are small compared with the differences in the average shear forces (Table 1). Correlations (n=24) also provided limited support for differences in shear force being attributable to variation in myofibrillar breakdown. Thus,

r(1d WB peak f).(1d MFI) = -0.04

r(20d WB peak f).(20d MFI) = 0.07

r(1d WB peak f).(1d KD30) = -0.44*

r(20d WB peak f).(20d KD30) = -0.26

The tendancy for higher peak forces to be associated with lower KD30 values at day 1 was supported by a significant negative correlation between the change in peak force from day 1 to day 20 and the change in MFI over that period (r = -0.39*).

Table 2: The concentration of 9 myofibrillar proteins following I day of ageing and the change in concentration from day 1 to day 20 of ageing.

Myofibrillar protein(s) (μg/mg myofibrillar protein)	Abbrev	After 1 day ageing			Change: 1 to 20 days		
		Low	Inter	S[a]	Low	Inter	S[a]
Myosin heavy chain	MHC	156.1	145.8	31.1ns	21.5	-9.3	35.2*
Alpha actinin	AA	24.0	19.0	5.4*	-2.6	-3.7	6.6ns
Actin	ACT	132.0	112.7	17.8*	5.0	-20.0	26.7*
Troponin-T	TROT	21.0	16.9	7.9ns	6.4	-3.9	6.6ns
26-37 kDa proteins	KD30	20.0	14.9	5.7*	-6.7	-5.9	9.1ns
Myosin light chain 1	MLC1	16.6	14.1	4.1ns	3.0	-3.2	6.1ns
Troponin-I & -C	TRIC	11.6	9.5	3.8ns	0.7	-1.7	4.7ns
Myosin light chain 2	MLC2	23.6	22.9	4.0ns	2.9	4.9	6.7ns
Proteins less than 18kDa	LT18	6.1	3.3	6.3ns	-1.6	-5.9	6.9ns

[a] See footnote to Table 1

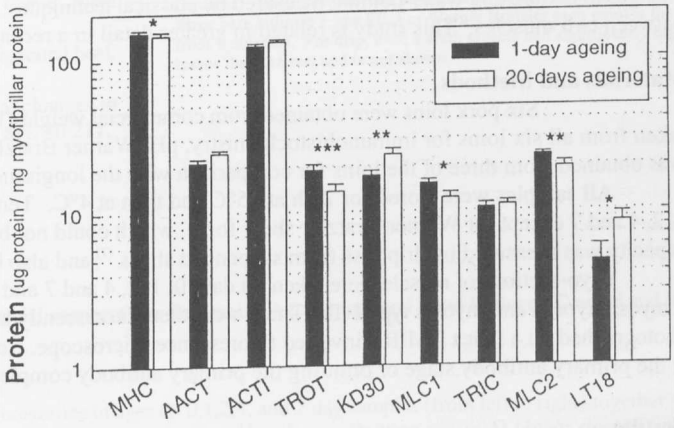

Figure 2: Concentration (on a log scale) of the 9 myofibrillar proteins defined in Table 2, for samples at 1 and 20 days ageing.

The ageing effect on the 9 myofibrillar proteins (Figure 2) is generally consistent with other reports (reviewed by Koohmaraie 1992) in showing that TROT concentration declined and that of KD30 proteins increased with ageing.

Myofibrillar protein levels given in Table 2 and Figure 2 are the averages of values obtained from SDS-PAGE bands that received a 2.5μl and a 5μl load. A measure of the linearity of the quantification method of these two loads is provided by results in Figure 3, where it is shown that the quantity of BSA estimated by densitometry increased at a slightly decreasing rate as the amount added to the myofibrillar extract increased from 0 to 1.8μg/ml, and that the relationship was similar when a 2.5 or 5.0μl load was applied. Over the two BSA levels used in this study (0.5 or 1.0μg) the relationship was close to linear. Estimates of the concentrations of myofibrillar proteins based on either the 2.5 or 5.0μl loads corresponded satisfactorily except that MHC levels were higher for the 2.5μl sample, presumably because the high concentration of this protein led to it being on the non-linear part of the curve.

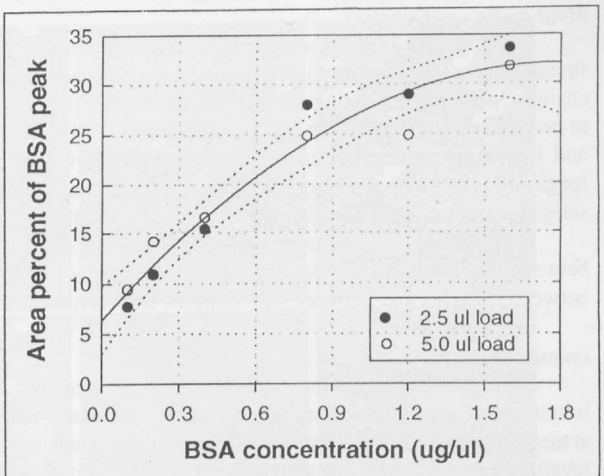

Figure 3: Increases in relative BSA peak area (from densitometry) with increasing BSA concentration (the quadratic regression line with 95% confidence intervals is shown; $R^2 = 0.95$)

In conclusion, it appears that, in this set of samples of beef longissimus muscle, there was very limited evidence to suggest that the greater toughness of the intermediate-pH samples was due to a lower level of myofibrillar protein breakdown over a 20-day ageing period.

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