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EFFECTS OF DOUBLE-MUSCLING ON BEEF TENDERNESS AND MYOFIBRILLAR FRAGMENTATION IN BELGIAN BLUE WHITE BULLS

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INTRODUCTION

Belgian Blue White (BW) cattle, showing signs of muscle hypertrophy are famous for their high meat yielding carcasses, resulting in significant higher returns at slaughter. However, little information is available on the meat quality of DM. DM muscle has decreased levels of collagen (Bailey *et al.*, 1982) implying a lower background toughness and therefore assumed to result in more tender meat (Boccard, 1981). Negative aspects of DM meat include pale colour, less taste and reduced water-binding (Boccard, 1981). In this study we have investigated the effects of double-muscling on meat texture, myofibrillar fragmentation and postmortem levels of calpains and cathepsins B & L.

MATERIAL AND METHODS

Animals

For this experiment, 59 Belgian Blue White bulls with normal conformation (20 months) and 32 double muscled bulls (20-22 months) were used.

Slaughtering and sampling

Animals were slaughtered after captive bolt stunning and pithing, and LD (8th thoracic rib) was subsampled at one hour post-mortem for protease determinations. At 24 hours post-mortem, LD steaks were removed, vacuum packed, aged for seven more days at 2°C, subsampled and finally frozen (-18°C) until analysis.

Sarcomere length

Sarcomere length (SL) was measured on three subsamples (±2g) of raw meat (eight days post-mortem), using laser diffraction (Vandendriessche *et al.*, 1984).

Shear force and cooking losses

The eight-day post-mortem steaks were used to determine cooking losses (weight differences upon cooking) and shear forces with a Warner Bratzler shear.

SDS-PAGE

Myofibrillar proteins (eight days post-mortem) were analyzed in a semi-quantitative manner, using Bovine Serum Albumin (BSA) as internal standard (CLAEYS *et al.*, 1993).

Intramuscular collagen

Collagen content (eight days post-mortem) was estimated by determination of hydroxy-proline concentration (ISO method Nr. 03496).

Extraction and assay of total cathepsins B & L (one and eight days post-mortem) + calpains (one and 24 hours post-mortem) was carried out as described by Uytterhaegen *et al.* (1992a).

RESULTS AND DISCUSSION

Global average LD shear force at 8d pm for all BW animals (n=91; DM + N) was 46 ± 15 Newton (mean ± S_D), indicating that the BW breed gives very acceptable tenderness when compared to other breeds. Crouse *et al.* (1991) detected 46N (six days post-mortem) for 15 month old Charolais. Wheeler *et al.* (1990) found seven-day post-mortem shear forces of Hereford and Brahman beef to be 65 and 78N respectively. However, variation coefficients of our shear force measurements within N and DM groups were about 24% illustrating again the typical variation in meat tenderness found within homogenous animal groups (Buts *et al.*, 1986b). Results of table 1 indicate that within the BW breed, LD shear forces at eight days post-mortem were significantly higher ($P<0.001$) in DM, although sarcomere lengths at this time were not different. As already previously reported (Buts *et al.*, 1986a; 1989), shear force was both in N and DM related significantly to sarcomere length ($P<0.002$), indicating that muscle contraction status accounted partly for shear force variation. Intercepts of the shear force-sarcomere length regression were found to be significantly higher in DM animals ($P<0.05$), whereas slopes were not statistically different (not shown). Cooking and drip losses (Table 1) were significantly higher in DM ($P<0.01$) confirming earlier results (Boccard, 1981).

Intramuscular collagen content (Table 1) was significantly reduced by 40% in DM ($P<0.001$), indicating that a lower background toughness could be expected in DM. The widespread vision that DM gives more tender meat than N because of a lower collagen content (Boccard, 1981; 1982; Bailey *et al.*, 1982; Bouton *et al.*, 1982) is not supported by these findings. However, Bouton *et al.* (1982) did not find a statistical difference ($P<0.05$) in Warner-Bratzler peak and initial yield force between *semitendinosus* muscles of differing biological types. Moreover, overwhelming evidence exists that tenderization is brought about mainly by degradation of myofibrillar proteins, probably by calpains (Goll *et al.*, 1991; Koohmaraie 1988; 1992; Uytterhaegen *et al.*, 1992b). So it is not surprising that reduced collagen contents of DM *longissimus* muscle do not result in more tender meat. Tremendous differences in post-mortem protease levels were found between N and DM (Table 2). Calpain 1 concentrations were significantly lower in DM at one hour and 24 hours post-mortem ($P<0.001$), as well as calpastatin capacity at one and 24 hours post-mortem ($P<0.001$) and were diminished considerably between one and 24 hours post-mortem. Extent of calpain 1 decline (due to autolysis) during that period, suggests a threefold higher preceding activity in N. Calpain 2 activity was not different at one hour post-mortem, but post-mortem cytosolic Ca^{2+} concentrations are considered to be too low for calpain 2 to be fully activated.

Calpain 1/calpastatin ratio at one and 24 hours post-mortem was significantly reduced in DM animals ($P<0.05$) denoting as well a decreased effectivity of calpain 1. In recent years evidence has accumulated that calpains are the main proteases involved in tenderization (Koohmaraie, 1988; 1992; Dransfield *et al.*, 1992; Dransfield, 1992; Goll, 1991) so calpain 1 induced lower levels of post-mortem proteolysis which could have been involved in causing texture differences. Remaining calpain 1 levels and calpain 1/calpastatin ratio's at 24 hours post-mortem indicate that post-rigor tenderization should be higher in N than in DM. The non-autolytical total (=free+bound) cathepsin B and L activities at one and eight days post-mortem were also significantly lower in DM ($P<0.001$). Total cathepsin B&L levels do not give an indication of the amount of "free" cathepsins, as these enzymes must be released from the lysosomes before they

can eventually degrade myofibrillar proteins and influence tenderness. However, the role of cathepsins B&L in post-mortem tenderization is generally considered to be far less important compared to calpains. Another interesting finding is that reported differences in cysteine protease levels (both calpain 1 and cathepsins B&L) are likely to be found back in muscle of living animals. Calpains and/or cathepsins are supposed to be involved in muscle protein turnover (Goll *et al.*, 1985; 1989; 1992). Calpains for initiating turnover by disassembly of myofilaments, whereas cathepsins are responsible for consecutive breakdown into shorter peptides and even amino-acids. So it could be that higher muscle yield in double muscled animals is associated with reduced protein turnover as a consequence of decreased protein degradation derived from decreased activities of these cysteine proteinases. These findings disagree with Boccard (1981) who suggested that muscle hypertrophy originates from disturbances of the collagen metabolism, which would lead to decreased restraints of surface muscles during their development.

However, available proteolytical potential seems to be limited in DM, compared to N. Lower background toughness in DM seems to be largely compensated for by decreased myofibrillar tenderisation, resulting in tougher meat. Texture differences were accompanied by reduced myofibrillar fragmentation in DM, as apparent from the significantly higher Titin levels ($P<0.01$) and the lower 30KDa concentrations ($P<0.05$), whereas Troponin-T concentrations were not different (Table 3). Troponin-T degradation is a well documented indicator for general proteolysis (Buts *et al.*, 1986a; Koohmaraie 1988; 1992; Ouali 1990; 1992; Uytterhaegen *et al.*, 1992a; 1992b) although in some research such significant positive relation with shear force was not found (George *et al.*, 1980; Salm *et al.*, 1983). Shear force and Troponin-T were related significantly in N ($P=0.000$), but this relation was not at all valid for DM at eight days post-mortem. Although Troponin-T was degraded to the same extent in both N and DM, LD shear forces appeared to be higher in the latter. However, Troponin-T has no role in structurally stabilizing the myofibrillar structure (Ouali, 1990) and up to now the "key" myofibrillar protein(s), responsible for tenderization is (are) still unknown. Possible candidates herefore include [?]-actinin, desmin and probably even other proteins which are present in minor amounts. As remaining Troponin-T concentrations at eight days post-mortem are no indicator of overall tenderness in DM, this might as well suggest that proteolytic tenderization is limited in DM meat. The very significant relation in N between WB shear force and 30KDa ($P=0.000$), the latter suspected to be a Troponin-T degradation product, is absent with DM animals. The relation between Troponin-T and 30KDa remains significant for DM, although absolute values for both slopes and intercepts were decreased very significantly ($P<0.001$). The well-known faster pH decline in DM is reflected by increased levels of denatured CPK (43KDa) and another two denatured sarcoplasmic proteins in the myofibrillar fraction, here called 34KDa and 35KDa components (Table 3). In previous research (Salm *et al.*, 1983; Uytterhaegen *et al.*, 1992a) was found as well that a faster pH-decline caused by electrical stimulation, yielded more of 43KDa and 34KDa peptides in SDS-PAGE patterns of myofibrillar proteins. Filamin concentrations were elevated in N ($P<0.01$), but it is not known if this protein has any role in post-mortem tenderization.

CONCLUSION

Further selection towards extreme muscling within the BW breed might result in more superior carcass quality but might have nevertheless adverse effects on meat quality, especially a decreased tenderness and diminished water binding. Reduced background toughness in DM is compensated for by higher myofibrillar toughness, probably caused by a deficient proteolytic tenderization, increasing the impact of rigor shortening. Muscle hypertrophy might originate from decreased muscle protein turnover, caused by reduced protein degradation.

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Table 1. Comparison of pH_{24} and LD meat quality characteristics of normal and double muscled Belgian Blue White bulls at eight days post-mortem.

	N	DM	sign.[1]
shear force (N)	38.3 ± 9.3	59.8 ± 14.1	***
sarcomere length (μ)	1.74 ± 0.10	1.80 ± 0.16	NS
cooking losses (%)	25.6 ± 2.8	30.0 ± 2.3	***
collagen (%)	2.50 ± 0.42	1.61 ± 0.16	***
drip losses (%)	5.4 ± 2.6	7.2 ± 1.5	***
pH_{24}	5.49 ± 0.13	5.54 ± 0.04	***

1: significance level of unpaired t-test; $P<0.05$ = *;
$P<0.01$ = **; $P<0.001$ = ***.

Table 2. Comparison of post-mortem enzyme levels of normal and double-muscled Belgian Blue White bulls.

	N	DM	sign. level[1]
calpain 1[3] (1 h pm)	29.4 ± 8. 7 (18)[2]	9.5 ± 2.8 (10)	***
calpain 1[3] (24 h pm)	9.0 ± 3.6 (8)	2.2 ± 1.2 (10)	***
calpain 2 [3] (1 h pm)	33.4 ± 6.0 (18)	31.1 ± 5.7 (10)	NS
calpastatin[3] (1 h pm)	1062 ± 183 (18)	470 ± 109 (10)	***
calpastatin [3] (24 h pm)	708 ± 262 (8)	347 ± 71 (10)	***
calp. 1/calpastatin (1 h pm)	0.0296 ± 0.014 (18)	0.0201 ± 0.03 (10)	*
calp. 1/ calpastatin (24 h pm)	0.0145 ± 0.009 (8)	0.0063 ± 0.003 (10)	*
total cathepsin B [4] (24 h pm)	62.5 ± 10.2 (8)	26.6 ± 6.7 (32)	***
total cathepsin B [4] (8 d pm)	51.7 ± 11.1 (59)	30.1 ± 9.1 (32)	***
total cathepsin L [4] (24 h pm)	84.9 ± 30.2 (8)	35.6 ± 14.1 (32)	***
total cathepsin L [4] (8 d pm)	87.9 ± 23.3 (59)	39.6 ± 16.1 (32)	***

[1] significance level of unpaired t-test; $P<0.05$ = *; $P<0.01$ = **; $P<0.001$ = ***.
[2] number of sampled animals.
[3] expressed as units activity/g muscle.
[4] expressed as nmol NH_2MeC/min/100g meat

Table 3. Comparison of myofibrillar protein concentrations at eight days post-mortem (mean[1] ± SD) of normal and double-muscled Blue White bulls.

SDS-PAGE myofibrillar fraction [1]	N n=59	DM n=32	significance level[2]
Titin	26.9 ± 4.8	31.4 ± 4.3	***
Filamin	1.8 ± 0.7	1.4 ± 0.8	**
43 KDa (CPK)	13.1 ± 4.9	16.8 ± 6.6	**
Troponin-T	1.3 ± 1.8	0.9 ± 0.8	NS
35 KDa	6.4 ± 1.4	7.3 ± 2.3	*
34 KDa	12.4 ± 3.9	16.5 ± 4.1	***
30 KDa	12.5 ± 3.0	10.1 ± 3.4	***

[1] expressed as μg BSA equivalents/mg myofibrillar protein
[2] significance level of unpaired t-test; $P<0.05$ = *;
$P<0.01$ = **; $P<0.001$ = ***.