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Relation between concentration of troponin-T, 30000 dalton, and titin on SDS-page and tenderness of bull Longissimus dorsi.

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Introduction

Post-mortem changes in bovine muscles have been associated with meat tenderisation (Goll et al, 1964) and have been related to troponin-T (TNT) degradation and the appearance of a 30000 dalton component on Sodiumdodecylsulphate poly-acrylamide gel electrophoresis (SDS-page) (Mac Bride & Parrish, 1977). These changes are related to enzymatic activity, especially to a calcium activated neutral protease (CaNP) (Cheng & Parrish, 1977; Locker et al., 1977; Parrish et al., 1981; Elgasim et al., 1985 ;Olson et al, 1977)
More recently the degradation of the newly discovered "gap-filaments" has also been implicated as being responsible for increasing meat tenderness (Locker et al., 1977 ; Locker & Wild 1984,a,b). Especially the high molecular weight (MW) myofibrillar protein titin has been considered as being highly succeptible to proteolysis (Lusby et al., 1983).
By using Bovine Serum Albumin (BSA) as an internal standard Penny and Dransfield (1979) have been able to elucidate a relation between the change in troponin-T and the change in toughness during ageing of beef.
But up till now no direct relation between concentration of TNT, 30000 dalton and titin separated by SDS-page and beef tenderness has been demonstrated. It is clear that the difficulties to obtain reliable estimates for protein concentration from SDS-page are a main obstacle in this respect.
In the present work we have succeeded to obtain semi-quantitive data expressed as BSA-protein (internal standard) equivalents for SDS-page separated myofibrillar proteins. Using this technique we have studied the relation between tenderness of beef longissimus dorsi and concentration of troponin-T, 30000 dalton protein, titin heavy band as well as sarcomere length within a group of similar animals.

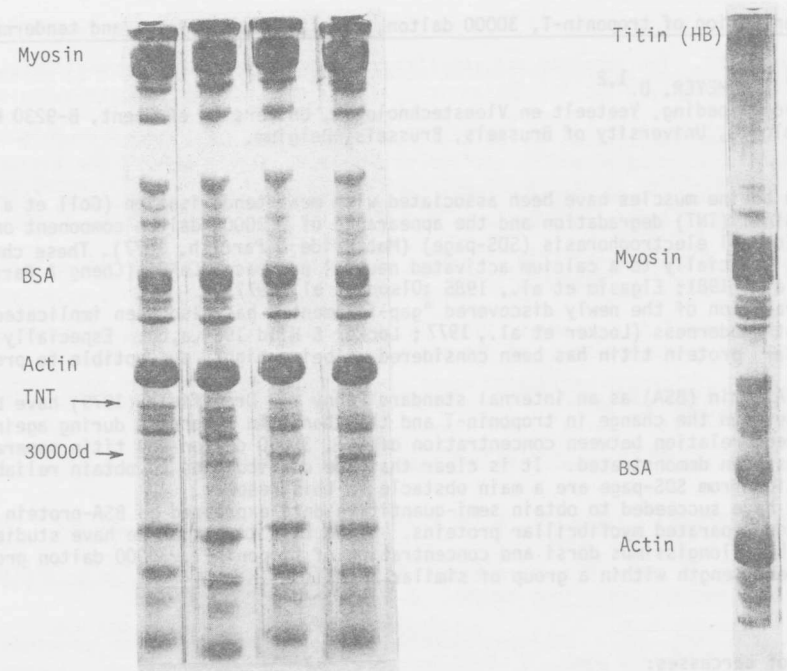
Material and Methods

Animals and treatment of carcasses:
Eighteen one year old bulls (mean value ± SE for live weight 465 ± 7 kg and dressing % 61,4 ± 0,5%) were slaughtered after captive bolt stunning and pithing in the slaughterhouse of our laboratory. Left and right carcass sides were subjected to a different cooling regime to induce differences in tenderness. Of each side (36 in total) the longissimus dorsi (8th thoracic rib) was removed 24h post-mortem (p.m.), vacuum packed and cooled (4°C) during 7 days. Eight days p.m. these cuts were subsampled for extraction of myofibrillar proteins and sarcomere length (SL) determination. Warner-Bratzler peak shear force values (WBS) were determined the same day after cooking of the 2,5 cm thick cuts.

Methods:
Warner-Bratzler peak shear force (WB-shear mounted on a Instron 1140, Instron ltd, High Wycombe) were determined perpendicular to the fibre direction on 1,27 cm diameter cork-bore samples, obtained from the cuts after heating. The cuts were heated in open plastic bags by immersion (1h) in a waterbath at 75°C and bags were cooled subsequently under running tap water to room temperature. Measurements of 15 to 30 cores were averaged.
Sarcomere Length (SL) was determined on fresh subsamples (ca 10 g) fixed in 2,5 % glutaraldehyde using laser diffraction as described by Vandendriessche et al. (1984). Average values of measurement of 20 different fibers per sample were obtained. Of about 10 g of each cut, myofibrillar proteins were isolated according to the procedure described by Parrish et al. (1973) using an Ultra turrax (type 18/10, Janke and Kunkel, KG Staufen).
Isolated myofibrils were dissolved overnight (room temperature, magnetic stirring) in Imidazole (0,01 M) Buffer (pH = 7,0) containing 2 % SDS and 2 % 2-mercaptoethanol (ME).
Solutions were then filtered to remove connective tissue (Schleicher and Schüll nr. 597 1/2 Filter). After determination of protein concentrations (micro-Kjeldahl), solutions were diluted to obtain 4 mg crude protein/ml and BSA (internal standard, Boehringer Manheim Gmbh, nr. 238031) was added (0,2 mg/ml).
After addition of bromophenol blue (ca 3 mg/10 ml) and ca 1,5 g sucrose/10 ml the solution is frozen and preservated at -18°C until electrophoresis.
Electrophoresis was carried out in an Acrylophor model 144 apparatus (Pleuger, Antwerp) using gel rods of 15 cm length and 6 mm diameter. Gels were always prepared the day before use. For the separation of troponin-T and 30000 dalton protein 10 µl of each protein solution was applied in duplicate on top of a gel with total acrylamide concentration (T) of 8 % and crosslinking (C) 3.1 % containing 0.55 % SDS and 0.50 % ME. For the separation of titin 25 µl of each solution was applied in duplicate on top of less concentrated gels (T = 4.58 %, C = 3.1 % containing 0.55 % SDS and 0.5 % ME).
After preconcentration of the gels, they were run at constant current (5 mA/gel) until the bromophenol blue front has traveled 10 cm from the gel top. Gels are then fixed by immersion in water containing 10% v/v acetic acid and 20% v/v methanol for 2 hours. Immersion of the gels in a 20 % ethanol - 10 % acetic acid aqueous solution during 3 hours washes out SDS.
Gels are stained overnight in an aqueous solution of Coomassie brillant blue R 250 (0.1 %) methanol (20 %) and acetic acid (10 %).
Destaining is done by immersion in the fixing solution until background colour is practically removed. Finally gels are scanned using a Beckmann (Model R-112) densitometer.
Peak areas were determined and protein concentrations (expressed as BSA protein equivalent) calculated as follows

$$\text{µg proteins/mg total myofibrillar protein} = \frac{\text{peak area protein band}}{\text{peak area BSA-band}} \times 50$$

Figure 1 shows typical separations obtained using concentrated (T = 8 %) and less concentrated (T = 4.58 %) gels. It should be noted that several bands can be distinguished between Actin and Tropomyosin. Troponin-T in our separation has a calculated molecular weight of 40,000, similar to separation patterns recently obtained by Salm et al. (1984) and Baumann et al. (1984).



TNT[1]	12.4	9.5	4.0	1.3
30000d[1]	2.6	5.4	9.1	13.5
WBS(N)	68.6	54.1	48.4	33.6

[1] µg BSA eq/mg myofibrillar protein

Figure 1 : Separation on concentrated (T=8%,left) and on less concentrated (T= 4.58%,right) gels.

Results and Discussion

Table 1 shows mean and range (min-max) for WBS, SL and protein concentrations, as well as the correlation matrix for these parameters.

Table 1 : Mean values (± SD), range and correlation matrix for WBS, SL and TNT, 30000 dalton and titin concentration.

	WBS (N)	TNT[1]	30000 dalton[1]	Titin HB[1]	SL (µ)
WBS	1	0.8064*	-0.7760*	0.5187*	-0.2311
TNT		1	-0.9335*	0.5071*	0.0411
30000d			1	-0.5058*	-0.0743
Titin				1	-0.0728
SL					1
Mean	52 ± 12	7.3 ± 3.8	9.4 ± 4.3	24.7 ± 2.0	2.00 ± 0.18
Range (min -max)	32 - 76	1.5 - 15.9	2.2 - 18.1	19.4 - 27.7	1.54 - 2.47

[1] concentration µg BSA equivalent/mg myofibrillar protein.

Level of significance : * : at least $p < 0.05$

These results indicate that within a group of similar animals tenderness and protein degradation are closely associated and confirm earlier results obtained by Olson & Parrish (1977) for the relation tenderness/TNT and 30000 dalton.
It must be noted that the concentration of other protein bands (tropomyosin and filamin) are also differing between animals but these concentrations are not correlated with WBS. Therefore the present work only dicusses TNT, 30000 d and titin for which a significant correlation with WBS is found.
A highly significant negative correlation is found between TNT and 30000 dalton concentration (Table 1) which

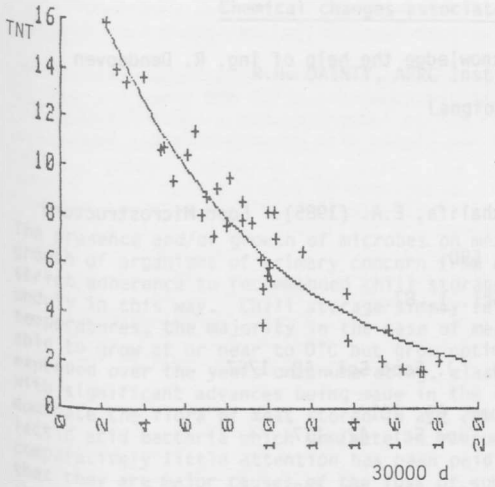


Fig 2 : Relation between 30000 dalton and TNT concentrations (µg BSA equivalents/mg myofibrillar crude protein)in bovine LD.

may indicate the 30000 dalton protein is a degradation product of TNT. A high correlation does not necessarily reflect a direct cause/effect relationship, however. Further analysis of the relation between TNT and 30000 d protein shows an exponential relationship (Fig. 2) best described by the following equation :

$$TNT_{conc.} = 20.42\ e^{-0.121(30000d)_{conc.}} \text{ with } r^2 = 0.902$$

The deviation from linearity can be an indication of an indirect relationship.
From the correlation matrix (Table 1) it is clear that WBS is also related to SL whereas the latter bears no relation to protein concentrations. This enables calculation of multiple regressions equations, introducing SL as second independent variable. This gives the following equations (Fig. 3, Fig. 4) :

$$WBS^{*} = -0.233(30000\ d)_{conc.} - 2.022\ SL + 11.650 \quad (r^2 = 0.69)$$

$$WBS^{*} = 0.272\ (TNT)_{conc.} - 1.860\ SL + 7.140 \quad (r^2 = 0.72)$$

In both cases, the introduction of SL allowed explanation of a significantly higher part of variability, than protein concentration alone. The relation WBS/titin (HB) (Table 1) is somewhat unexpected because King et al.(1981) indicated that connectin (shown to be identical to titin by Maruyama et al.,(1984) is degraded very fast during heating of meat which makes a contribution to meat toughness most unlikely. Lusby et al. (1983) have found that the intensity of the titin bands is also decreasing during post-mortem storage. We have found a very important degradation of titin both during ageing and heat treatment (unpublished results) of beef longissimus dorsi. The finding that titin concentration is correlated with WBS as well as with TNT and 30000 dalton protein concentration (Table 1) may indicate that both titin and TNT concentration reflect overall myofibrillar and cytoskeletal protein integrity contributing to tenderness. Both proteins, as well as the 30000 dalton compound formed are probably not directly related to shear force or tenderness in bovine LD.

* WBS in Kg

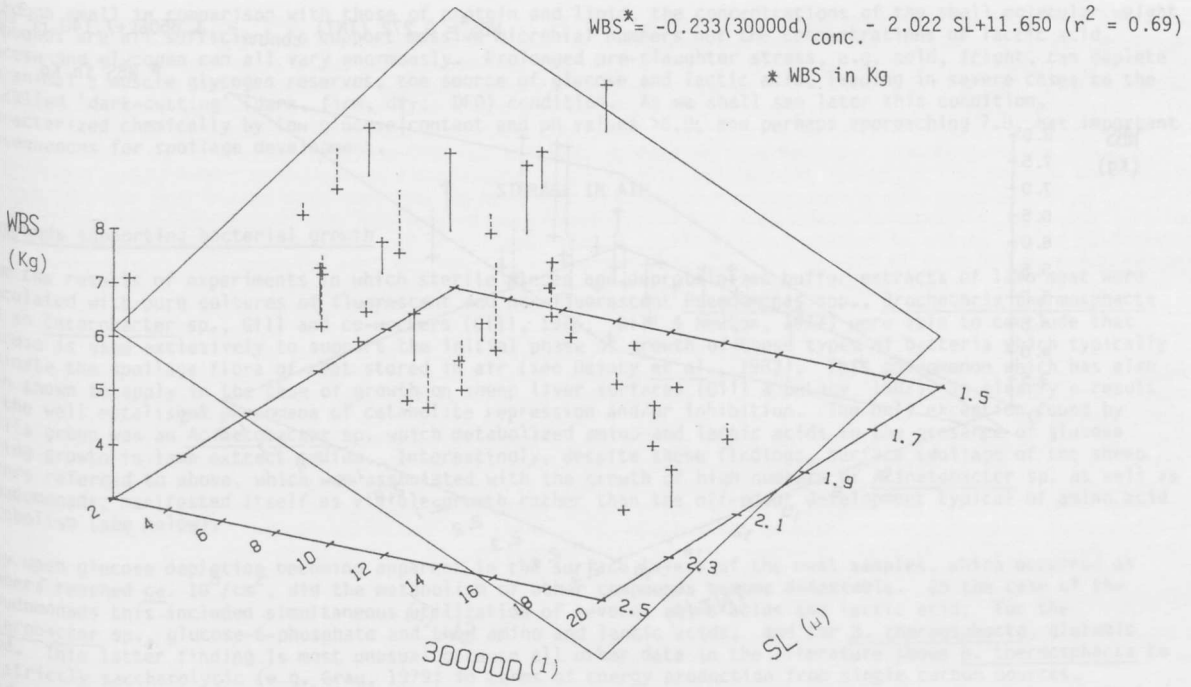


Figure 3 : Lineair regression between WBS (dependent variable) and 30000 d and SL (independent variables

——— above the plane
----- under the plane
1 µg BSA eq/mg myofibrillar protein

Acknowledgements

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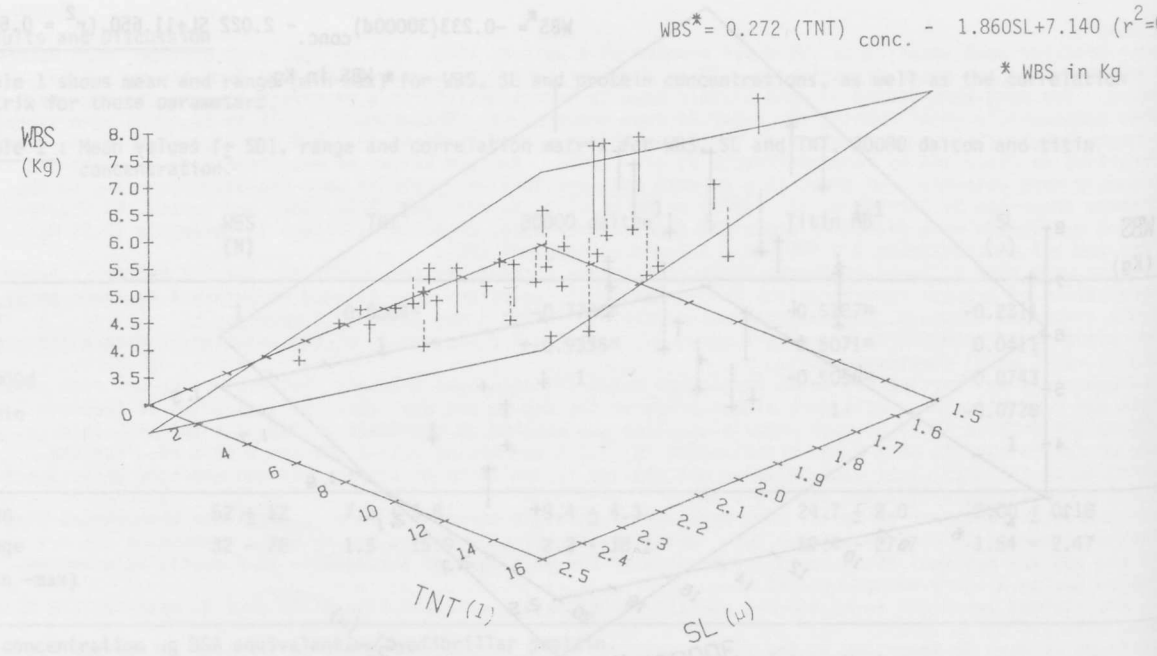

Figure 4 : Lineair regression : between WBS (dependent variable) and TNT and SL (independent variables).

1 µg BSA eq/mg myofibrillar protein