CALPAIN SYSTEM EXPRESSION IN THREE SOUTH AFRICAN BEEF CROSS-BREEDS

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Introduction

One of the most important meat quality attributes of meat is meat tenderness (Ouali, 1991). The calpain system expression in three typical South African cross breeds (Brahman-crosses, n=20, Nguni-crosses, n=20 and Simmentaler-crosses, n=20) was studied by means of SDS-PAGE and Western-blotting. The degradation of proteins such as titin, nebulin, and desmin have shown to play a role in the development in meat tenderness (Huff-Lonergan et al. 1996) and act as substrates for the calpain system known to be involved with the *post mortem* ageing of meat. The purposes of this paper is to illustrate genetic expression differences of the calpain system between typical South African *Bos indicus, Sanga* and *Bos taurus* genotypes.

Materials and Methods

60 Simmental cross, Brahman cross and Nguni cross bulls were raised, treated and slaughtered under intensive conditions and aged 12 months,. Non stimulated carcasses were chilled directly after dressing at room temperature before loading at 0 – 4°C. Sampling of the *M. longissimus* (LD) for measurement of Warner Bratzler shear force (WBS), calpain system, and SDS-PAGE/Western blotting were taken at 1 hour and 24 hours *post mortem.* LD of both sides were sampled. Samples destined for WBS, and SDS-PAGE/Western blotting were vacuum packaged and aged at 2°C ± 2 ºC for 1, 7 and 14 days *post-mortem.* LD aged for 1, 7 or 14 days were processed into 30 mm steaks by means of a band saw before being thawed at 4°C for 24 h and prepared according to an oven-broiling method using direct radiant heat (AMSA, 1978). The steaks were broiled at 260°C (pre-set) to 70°C internal temperature. Core samples of 12.5 mm were removed along the fibre and sheared perpendicular to the fibre with a Warner Bratzler shear device attached to an Instron Universal Testing (Instron, 1990). Shear force was measured as the peak force (kg) average for eight cores per sample. Calpains and calpastatin were extracted and measured (Frylinck *et al*., 2003). One unit of calpain activity was defined as an increase in absorbance at 366 nm of 1.0 per hour, at 25 °C. One unit of calpastatin activity was defined as the amount that inhibited one unit of m-calpain activity. To study the degradation of titin, nebulin, desmin and appearance of the ~30 kDa degradation products as a result of tenderization the LD proteins from samples taken 1, 7, and 14 days *post mortem* were separated using 30%T 0.5%C SDS-PAGE separation gels with 12%T, 12.5%C stacking gels (Fritz *et al.*, 1989). The separated muscle protein bands by means of SDS-PAGE electrophoresis were analyzed densitometrically with the help a video image analyzer equipped with the ImageMaster 1D Software (Amersham Pharmacia Biotech). Desmin's transfer and Western blotting procedures were done according to the procedure of Huff-Lonergan (1996). The electrophoresis and Western immuno-blot patterns were analyzed densitometrically with the ImageMaster 1D Software (Amersham Pharmacia Biotech). The data were subjected to a three-way analysis of variance. Means for the main effects and their co-variants were separated using Fisher's protected t-test least significant difference (LSD) at the 5% level.

Results and Discussion

In this study the Brahman and Nguni loins were similar in tenderness, but significantly more tender than Simmentaler loins. The calpain system and its expression did not explain these results; the calpastatin/µ-calpain ratio measured at 1 hour and 24 hours *post mortem* was significantly lower for both Nguni and Simmentaler crosses compared to Brahman crosses. The degradation (diminishing of intensities of SDS-PAGE/Western blot protein bands) of titin, nebulin, and desmin (substrates of µ-calpain) was consistently more effective in the Simmentaler cross than in the Nguni or the Brahman crosses (Table 1). Titin and desmin degradation was significantly slower in the Nguni cross compared to the Simmentaler cross and that of the Brahman cross not significantly different from the other two breeds. The same trend (P=0.071) was found for nebulin degradation. The production of the 30 kDa protein fraction did not differ significantly between the different breed-crosses.

Although titin and desmin seem to degrade slower in the Nguni cross, overall it seems that the calpain system expression is similar to that of the Simmentaler cross. It is known that Bos indicus has higher calpastatin levels than that of the Bos Taurus (Shackelford *et al*., 1995) and this fact corresponds with the higher calpastatin/ µ calpain ration measured in the Brahman than in the Simmentaler. If only the calpain system characteristics were taken into account the Simmentaler and Nguni crosses should have been the most tender groups, and the

Brahman cross the toughest. Clearly other factors must have influenced the tenderness characteristics in this group of animals tested.

Table 1. Effect of breed on tenderness and the calpain system expression[1].

	Brahman-X	Simmental-X	Nguni-X	SEM	p-Value
WBS (N/1.25 cm θ)	6.33[a]	7.30[b]	6.53[a]	0.21	< 0.003
Calpastatin / μ-Calpain ratio					
1 hour *post mortem*	1.72[b]	1.33[a]	1.28[a]	0.083	< 0.001
24 hours *post mortem*	2.26[b]	1.59[a]	1.42[a]	0.106	< 0.001
Titin (~ 300 kDa) [2]	0.34[ab]	0.31[a]	0.37[b]	0.016	< 0.033
Nebulin (~ 250 kDa) [2]	0.04	0.03	0.04	0.004	0.071
Desmin (~55 kDa) [2]	8.31[ab]	7.90[a]	8.53[b]	0.164	< 0.024
30 kDa [2]	0.058	0.054	0.061	0.014	0.474

[a,b] Means in the same row without a common superscript letter differ significantly ($P < 0.05$).
[1] Mean values for 1, 7, and 14 days ageing.

Conclusions

Because the tenderization of meat is a multi factorial event, it will not be enough to only measure the contribution of the tenderization mechanism involving the calpain system in order to predict its potential meat tenderness. New technologies must include the potential contribution of the other mechanisms, such as the level of contraction of actin and myosin, as well as the contribution of the connective tissue characteristics.

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