The influence of muscle characteristics and calpain genes on beef tenderness in South African feedlot cattle

G.L. van Wyk[1], J. Anderson[1] , L. Frylinck[1], P.E. Strydom[1], H. Snyman[1] , E.C. Webb[2], M. Koohmaraie[3] & T.P.L. Smith[3]

[1]Agricultural Research Council of South Africa, Private Bag X2, Irene, South Africa, 0062.
[2]Department of Animal and Wildlife Sciences, University of Pretoria, 0002.
[3]USDA, ARS, U.S. Meat Animal Research Center, Clay Center, NE 68933.
E-mail: louisam@arc.agric.za.

Abstract

Weaned, young bulls (n = 60) were selected on phenotype from various commercial producers to represent a Brahman (*Bos indicus*; n = 20), Simmental (continental *Bos Taurus*; n = 20) and Nguni (*Sanga*; n = 20) crossbred group. After being raised under intensive feedlot conditions the animals were slaughtered according to normal South African slaughter procedures. At slaughter the carcasses were not electrical stimulated because electrical stimulation influences the processes of meat tenderness, and the emphasis was on the expression of the inherent tenderness characteristics without external *post mortem* influences. A multiplex marker system incorporating markers for the *CAPN1* gene and the *CAST* gene were evaluated in this study as well as Warner-Bratzler shear force (WBSF), desmin degradation (SDS-PAGE, Western-blotting), myofibril fragment lengths (MFL), sarcomere length (SL) and the calpain system. The results of the study showed that differences exist in meat quality of the different crossbreds. In general, the animals in this study had the tendency for tougher meat. The aim of this study was to investigate the relationship between various crossbreds and muscle characteristics, considering a physiological and genetic approach.

Introduction

The concept of meat tenderness is regarded as a multi-factorial process as it is biologically dependant on a combination of many genetic and physiological factors. The development of molecular theory provides new strategies for improvement of meat tenderness through a genetic approach (Koohmaraie *et al*, 2003) that is a non-invasive method and can be applied while the animal is still alive. Currently, the biochemical pathway with the most evidential support for involvement in *post mortem* tenderization is that of the calpain family of proteases. Two enzymes responsible for this process are the micro molar calcium-activated neutral protease µ-calpain (*CAPN1-4751*), which is encoded by the *CAPN1* gene, which is found or situated on bovine chromosome 29. Its inhibitor, calpastatin (*CAST*), which is found on chromosome seven, is encoded by the *CAST* gene (Koohmaraie, 1996). These markers can be associated with a measure of beef tenderness i.e. WBSF (Page *et al*, 2004; White *et al*, 2005; Casas *et al*, 2006). Certain breeds are genetically dispositional to produce meat that is tougher. It is well documented that tenderness decreases as the percentage *Bos indicus* increases in an animal (Crouse *et al*, 1989).

Material and methods

The position of sampling of the *M.longissimus* (LD) for each test was consistent and took place 24 hours *post mortem*. DNA extractions were performed with a commercial kit, DNeasy Tissue (QIAGEN). At U.S. Meat Animal Research Centre (MARC) the methods for determining *CAPN1* was based on the methods described by Page *et al*. (2002) and White *et al*. (2005), where the determination of *CAST* was based on the method of Casas *et al*. (2006). Genotypes for each animal were analyzed, and the automated calls were checked by manual visualization of the spectrographs to minimize errors. MFL and SL were measured using a Video Image Analyzer and tenderness was measured by WBSF measurements (Strydom and Frylinck, 2005). Desmin degradation was studied by means of SDS-PAGE electrophoresis (Fritz *et al*., 1989) and the transfer and Western-blotting procedures were carried out in accordance with the procedure of Huff-Lonergan *et al*. (1996). The electrophoresis and Western-blot patterns were analyzed densitometrically with the ImageMaster 1D-Software (Amersham Pharmacia Biotech). Calpains and calpastatin were extracted from frozen samples (Dransfield, 1996) and separated by means of a two-step gradient ion-exchange chromatography method (Geesink and Koohmaraie, 1999). Calpain assays were done using azo-casein as substrate and one unit of calpain activity was defined as an increase in absorbance at 366 nm of 1.0 per hour at 25 ˚C. One half unit of calpastatin activity was defined as the amount that inhibited one unit of m-calpain activity. The data were investigated by ANOVA and the genotype frequencies were calculated by direct count.

Results and discussion

Characteristics evaluated and simple statistics for the three crossbreds are presented in Table 1.

Table 1. Least square means and standard errors of means for the various characteristics evaluated in the three crossbreds

			Brahman-X	**Simmental-X**	**Nguni-X**
Characteristic	**P-Value**	**SEM**			
WBSF (kg)	< 0.003	0.21	6.33[a]	7.30[b]	6.53[a]
µ-calpain					
1 hour *post mortem*	< 0.005	0.13	1.68[a]	2.20[b]	2.22[b]
24 hours *post mortem*	< 0.016	0.10	1.48[a]	1.79[b]	1.91[b]
Calpastatin/µ-calpain					
1 hour *post mortem*	< 0.001	0.08	1.72[b]	1.33[a]	1.28[a]
24 hours *post mortem*	< 0.001	0.11	2.26[b]	1.59[a]	1.42[a]
MFL	0.641	1.36	34.84	36.30	34.64
Desmin (SDS-PAGE)	< 0.018	0.01	0.054[b]	0.046[a]	0.052[b]
Desmin (Western-blot)	< 0.024	0.16	8.31[ab]	7.90[a]	8.53[b]
Sarcomere length	< 0.035	0.02	1.70[b]	1.66[b]	1.61[a]

Significant differences ($p < 0.003$) were found in the overall WBSF values for the three different crossbreds. The Brahman-crosses were found to have significant lower shear force values (more tender) and the Simmental-crosses with the highest shear force values (less tender) with the Nguni-crosses in an intermediate position under these specific experimental conditions. On average, between 1 hour and 24 hours *post mortem* the Brahman-crosses had the lowest µ-calpain activity, which differed significantly from the other crosses evaluated. The Simmental-crosses and the Nguni-crosses were similar with the Nguni-crosses being higher on average, favoring the proteolytic *post mortem* tenderizing process. The calpastatin/µ-calpain ratio for the Brahman-crosses was significantly higher ($p < 0.001$) than that for the other crossbreds evaluated. No significant differences for MFL were found between the three different crosses, but the Simmental-crosses had a tendency towards longer lengths compared to that of the Brahman and Nguni-crosses. Desmin degradation determined via electrophoresis was similar between the Brahman- and Nguni-crosses. Desmin degradation evaluated by means of Western-blotting showed that the Simmental- and Nguni-crosses differed significantly, where the Brahman-crosses were intermediate to the Simmental- and Nguni-crosses. In this study the sarcomere lengths were significantly different between the crossbreds. The Brahman-crosses SL's were significantly longer (more tender) than SL's of the Nguni-crosses but similar to the Simmental-crosses. This is not unexpected as the Brahman- and Simmental-crosses carcasses were larger and thus less prone to muscle shortening.

Table 2 shows the number of animals with the genotypes for the SNP used in the *CAST* and *CAPN1* markers.

Table 2. Number of individuals inheriting the CC, CT, and TT genotypes at the calpastatin (*CAST*) and µ-calpain (*CAPN1*) genes in the three crossbreds

	CAPN1											
	Brahman-X				**Simmental-X**				**Nguni-X**			
CAST	CC	CT	TT	Total	CC	CT	TT	Total	CC	CT	TT	Total
CC	0	0	1	1	0	0	1	1	0	1	6	7
CT	0	1	4	5	0	3	4	7	0	2	6	8
TT	0	6	6	12	0	1	11	12	0	1	3	4
Total	0	7	11	18	0	4	16	20	0	4	15	19

Genotypes for the CC class at *CAST* and *CAPN1* had no frequency across the crossbreds (Table 2). The low frequency of animals inheriting the CC genotype at the *CAST* marker generated a low frequency of allelic combinations with *CAPN1*. For example, only 8 animals inherited the CC *CAST* and the TT *CAPN1* genotype in the three crossbreds. According to Casas *et al*, (2006) animals inheriting the CC genotypes at both markers were more tender. Most of the animals in this study presented the CT-TT and TT-TT genotypes. This corresponds with the WBSF values in Table 1, which indicated that overall the meat of the

animals of this study is tough. The question could be raised that if all the animals in this study had favorable genes, the environmental factors still have the potential to influence the animals in a positive or negative way?

Conclusions

Although the breed crosses differed phenotypically the animals represented in the crossbred groups could be genotypically more similar, because the contribution of the different breeds represented in the crosses could not be established on genotype (unknown). It can be concluded from the overall data (i.e. WBSF), that the animals in this study had tough meat in general. External factors and various mechanisms are still researched (current study at ARC) to determine the role these factors play on the final tenderness outcome and if any.

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