EFFECTS OF SNPs IN THE CALPAIN-1 AND CALPASTATIN GENES ON TENDERNESS IN SOUTH AFRICAN CROSSBRED BEEF CATTLE

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Abstract – Tenderness was evaluated in crossbred cattle (n=180) under South African production conditions. Bulls were finished on a grain diet and slaughtered to investigate the effects of calpain system genetic markers on tenderness. Two single nucleotide polymorphisms (SNPs) of the bovine calpain-1 gene (*capn1*) and two SNPs in the calpastatin gene (*cast*) were evaluated in three breeds, subjected to three different post-slaughter treatments (electrical stimulation). There were differences in the distribution of favourable alleles between breeds for SNPs, with a high frequency of favourable alleles in Nguni crosses. Both *capn* and *cast* SNPs (and/or their haplotypes) decreased Warner-Bratzler shear force (WBSF) during the ageing process up to 14 days by as much as 1.6 kg, while myofibrillar fragmentation length (MFL) at 14 days ageing was also improved by favourable alleles in these genes. The activity of calpastatin and calpain was poorly predicted by individual SNPs, while the power of prediction of enzyme activity was improved when the SNPs were combined haplotypes. These SNPs (and haplotypes) were suitable for selection for tenderness in South African beef cattle and with the use of these and potentially other SNPs associated with beef quality, could be useful tools for improving the quality of beef.

Key Words – capn-1, cast, South African beef, tenderness

I. INTRODUCTION

There is some unexplained variation in tenderness that occurs during the maturation process of meat that cannot always be accurately predicted and can vary significantly between breeds and peri-slaughter conditions [1]. Studies using South African beef cattle breeds, which can link a comprehensive set of genetic and/or biochemical markers to physiological processes in carcasses and eventual meat characteristics, is essential. In the first phase of this study, *Bos indicus* (sanga type and zebu type) crosses were compared to *Bos taurus* crossbred beef cattle to characterise the allelic distribution in South African cattle for commonly-used markers in the calpain system, as well as the association of these SNPs with tenderness, for use in selection.

II. MATERIALS AND METHODS

Crossbred (F1) bulls were finished by grain feeding at the ARC-Animal Production Institute in Pretoria and included 180 animals (n=60 of each breed) phenotypically classified as Brahman crosses (Bh-X, Zebu-type *Bos indicus*), Nguni crosses (Ng-X, Sanga-type *Bos indicus*) or Simmental crosses (Sm-X, continental *Bos taurus*). Prior to slaughter, animals were transported 50 km, penned with access to water and then slaughtered after stunning with captive bolt. Carcasses were suspended from the Achilles tendon and subjected to post-slaughter treatment, being non-stimulated (NS), stimulated for 15 seconds (ES-15) or stimulated for 120 seconds (ES-120) at 400 V peak, with 5 ms pulses at 15 pulses per sec. All carcasses were chilled directly at 4 °C. Samples from the *Longissimus lumborum* muscle (LL) were collected and LL steaks were aged 1, 7 or 14 days following vacuum packing. MFL was determined using 100 fragments per sample by means of Video Image Analysis according to the method of Culler [2] as modified by Heinze [3]. The calpains were extracted following 1 day and 20 days ageing, by 2-step gradient ion exchange chromatography.

For WBSF, frozen steaks (30 mm) were thawed at 4 ± 1 °C overnight for determination on a Universal Instron apparatus (Model 4301, Intsron Ltd, Buckinghamshire, England) after oven-broiling to 70 °C internal temperature using direct radiant heat [4]. The mean value of the load at maximum (kg) of 8 cores was used in statistical analysis, after elimination of outliers. Small samples of muscle tissue were also collected for the extraction of DNA using DNeasy® blood and tissue kit. Animals were genotyped using the primer extension method and a Mass Array system at the US Meat Animal Research Center (MARC). Genetic markers (SNPs) were located in the calpain-1 gene (*capn1-316* and *capn1-4751*) and calpastatin gene (*cast-C/T* and *cast-Bh*). The *capn1-316* (C>G) SNP is located in exon 9 (domain II) and is associated with a substitution of alanine ("tender allele") with glycine of the enzyme [5]. The *capn1-4751* SNP marker is associated with the substitution of the "tender allele", C (cytosine) with T (thymine) of intron 17 [6]. The *cast-C/T* SNP is a substitution of a C allele for a T ("tender") allele. The *cast-Bh* SNP is characterized by substitution of A (adenine, "tender") with T (thymine) in intron 5 [7]. Haplotypes were identified by assigning a number as the sum of favourable alleles, regardless of the genetic marker (specific SNP) from which these favourable alleles originated. Statistical analysis was performed with general linear model analysis and least significant difference using SAS [8], with $P<0.05$ to indicate statistical significance, unless stated otherwise.

III. RESULTS AND DISCUSSION

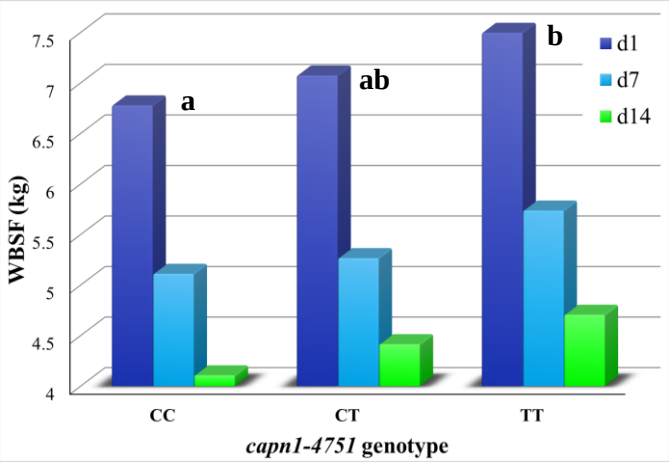
The favourable allele frequency for *capn1-316* is generally very low [9], although it is much higher in some populations of especially Angus cattle [10] [11]. It was relatively high in these crossbred cattle (Table 1), mainly due to the high frequency observed in Ng-X (33%). The allele frequency of Sm-X was lower than expected and could be the result of the *Bos indicus* influence of crossbreeding. The favourable allele frequency in *capn1-4751* was even higher, with more than 50% in Nguni cattle. For both the *cast* SNPs, the favourable allele frequency was very high and the low proportion of unfavourable alleles could limit the usefulness of these SNPs, especially *cast-Bh*, where no unfavourable homozygotes were observed. These markers could however still prove useful in haplotype analyses. The tenderness for Bh-X and Ng-X after 14 days ageing was generally improved by 5 - 10% (≃ 0.5 kg lower shear force) compared to Sm-X.

Table 1 Genotype and allele frequencies

Marker (SNP)	*Genotype Frequency*			*Allele Frequency*	
capn1-316	CC	CG	GG	C (favourable)	G
TOTAL	4.0%	20.5%	75.6%	14.2%	85.8%
Bh-X	1.7%	8.5%	89.8%	5.9%	94.1%
Ng-X	10.3%	44.8%	44.8%	32.8%	67.2%
Sm-X	0.0%	8.5%	91.5%	4.2%	95.8%
capn1-4751	CC	CT	TT	C (favourable)	T
TOTAL	12.6%	41.1%	46.3%	33.1%	66.9%
Bh-X	5.2%	31.0%	63.8%	20.7%	79.3%
Ng-X	28.8%	47.5%	23.7%	52.5%	47.5%
Sm-X	3.4%	44.8%	51.7%	25.9%	74.1%
cast	CC	CT	TT	T (favourable)	C
TOTAL	5.3%	35.5%	59.2%	76.9%	23.1%
Bh-X	8.6%	50.0%	41.4%	66.4%	33.6%
Ng-X	1.9%	20.8%	77.4%	87.7%	12.3%
Sm-X	5.2%	34.5%	60.3%	77.6%	22.4%
cast-Bh	AA	AT	TT	A (favourable)	T
TOTAL	93.4%	6.6%	0.0%	96.7%	3.3%
Bh-X	90.9%	9.1%	0.0%	95.5%	4.5%
Ng-X	100.0%	0.0%	0.0%	100.0%	0.0%
Sm-X	90.9%	9.1%	0.0%	95.5%	4.5%

Contributing to this breed difference could be the low number of Sm-X that are homozygous for the favourable *capn-1* alleles. Post-slaughter treatment (electrical stimulation) consistently decreased WBSF values by 20 - 40% (1.0 - 2.5 kg), with little or no difference in shear force values between 15 seconds *vs.* 120 seconds of stimulation. For *capn1-4751*, the homozygotes with 2 copies of the favourable allele (CC), exhibited a 7 - 10% improvement in shear force (0.4 - 0.5 kg) compared to TT, with intermediate tenderness for the heterozygous cattle (Fig. 1). These results are similar to Casas [12] and Cafe [13], where the SNP was also effective as part of a haplotype analysis, but more consistently found here. Although the *cast* SNPs failed to influence the WBSF values individually or as a *cast* haplotype, combination of *castC/T* with the calpain-1 haplotype was associated with a 1.6 kg reduction in shear force as the number of favourable alleles increased from zero to 6, although this was only significant on day 7 of ageing. This indicates the additive effects of markers in the calpain system on tenderness [14] and that these combined genetic markers could be more effective as selection tools than individual markers.

Figure 1. Association of *capn1-4751* genotypes with WBSF following 1 day, 7 days and 14 days ageing



The MFL after 14 days of ageing was significantly affected by post-slaughter treatment, *capn1-316* and the calpain-1 haplotype. The *capn1-316* CC genotype resulted in a 2.1 μm reduction in fragment length on day 7 and 2.9 μm on day 14 of ageing, compared to the GG genotype. An increase in the number of favourable alleles in the *capn-1* haplotype resulted in a significant decrease in the fragment lengths from 27.0 μm for 0 favourable alleles to 22.8 μm for 4 favourable alleles. Although *castC/T* itself did not affect MFL, a haplotype combining the two calpain-1 markers with *castC/T*, resulted in a significant decrease of MFL from 4.42 μm for animals with no favourable alleles, to 4.09 μm for 5 favourable alleles included in the haplotype. This indicates that *cast* SNPs were more effective predictors of tenderness as part of a haplotype than individually [15]. The activity of the calpastatin inhibitor after 1 or 20 days ageing was not different between breeds. It was inconsistently affected by the *castC/T* genotype, not by *cast-Bh* and affected by the *cast* haplotypes, which more accurately predicted calpastatin activity. The activity of calpain-1 was affected by breed, where the activity of the protease was significantly lower in Bh-X than Ng-X and Sm-X (approximately –20%), although this did not seem to compromise WBSF, as this was generally the breed with the most tender meat, although not statistically different from Ng-X. Although calpain-1 activity was not determined by the *capn1-316* genotype and inconsistently linked to *capn1-4751* genotype, it was increased by an increase in the number of favourable alleles in the calpain haplotype, reinforcing the concept that haplotype analysis is more efficient than individual SNPs.

IV. CONCLUSION

There is significant room for improvement of allelic frequencies of *capn-1* in South African beef cattle. There is a need for SNPs or a panel of SNPs to improve the predictive power for tenderness in South African beef, including the SNPs of the calpain system, but also alternative markers associated with the multifactorial processes that convert muscle to meat. Nguni cattle have the genetic potential to produce beef of superior tenderness, but due to their smaller carcasses also require more careful management of post-slaughter conditions to allow full development of the phenotype that is subject to a large environmental influence [16].

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