A Calpastatin Insertion/Deletion Polymorphism is Associated with the Myofibril Fragmentation Index in Bos taurus Bulls

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ABSTRACT

The objective of this experiment was to determine whether there was a difference between the Myofibril Fragmentation Index (MFI) of longissimus (LD), semimembranosus (SM) and semitendinosus (ST) muscle samples obtained from Senepol (n = 12) and Charolais (n = 12) crossed bulls. Also, the relationship between single nucleotide polymorphism (SNP) identified in the Calpastatin (CAST) gene locus and MFI was studied. Therefore, bulls were genotyped for a cytosine insertion/deletion identified at intron 2 of bovine CAST (allele A = cytosine deletion; allele B = cytosine insertion). Calves were weaned at 9 months (266.5 kg.) and then raised under grazing conditions until harvest at 22 months (499.6 kg.). For each bull, 2 subsamples of the three different muscles were cut in 1 cm² pieces and homogenized using a Polytron homogenizer (30,000 RPM) in cold (4°C) homogenizing buffer (100 mM KCl; 7 mM KH_2PO_4 ; 18 mM K_2HPO_4; 1 mM EDTA; 1 mM NaN_3 [pH to 7.0]). Muscle protein concentration was determined with the Biuret reagent assay. All muscle homogenates were diluted to a constant concentration of 0.5 mg of protein /mL of homogenization buffer. Subsequently, the absorbance of each muscle homogenate was determined at 540 nm. Absorbance readings were multiplied by 200 to determine MFI values.

There was no interaction between breed and muscle (P=0.9603). Moreover, MFI values were not affected by breed (P=0.1366). Also, no interaction between muscle and CAST-SNP was observed (P=0.9354). However, CAST-SNP tended to have an effect in MFI values. Specifically, bulls with the AA genotype tended to have higher MFI values than AB genotype (P=0.0912 when the muscle*CAST-SNP interaction is included in the lineal model; P=0.07 when such interaction is removed). Bulls with the AA genotype showed MFI values of 41.22 (s.e. ± 3.61) for LD; 24.17 (s.e. ± 2.22) for SM; and 35.86 (s.e. ± 3.24) for the ST muscle. Bulls with the AB genotype showed MFI values of 33.65 (s.e. ± 2.57) for LD; 20.37 (s.e. ± 2.17) for SM; and 35.86 (s.e. ± 3.24) for the ST muscle. If only data from the LD muscle was taken into account, the MFI values were not only different between CAST-SNP genotypes (P = 0.04) but also among crossbreds (P= 0.012). In such case, the MFI resulted higher in the LD of Senepol bulls. Because previous studies have associated higher MFI values with more tender meat, our data suggests that the CAST-SNP might be useful in animal selection due to its association with MFI in Senepol and Charolais crossbreds.

Key Words: MFI, beef tenderness, longissimus muscle, semitendonous muscle and semimembranous muscle

INTRODUCTION

Myofibrillar fragmentation (the extent of fragmentation of myofibrils caused by homogenization) has been shown to be highly correlated with indices of meat tenderness, such as Warner-Bratzler shear force and sensory tenderness (Moller et al., 1973; Olson et al., 1976). The progress of this tenderization can be assessed in the laboratory by measuring the fragmentation of myofibrillar proteins using a spectrophotometer. (Roberto D. Sainz, 2001) Meat tenderization results from the breakdown of muscle structure (myofibrils) after slaughter, a process mediated by naturally occurring muscle enzymes (Calpains) and inhibited by a protein called Calpastatin. The calpains are activated by calcium ions and have maximum activity in neutral to alkaline conditions (Warriss, 2000). Several technologies have been developed to overcome problems in meat tenderness, such as freezing and thawing (destroys calpastatin activity), calcium chloride injections and vitamin D feeding (increases calpain activity) and even hydrodynamic shock (physically disrupts the myofibril). So far, none of these techniques has been adopted by the packing industry because of high initial costs and uncertain benefits. Therefore, selecting beef cattle genetically predisposed to have tender meat would greatly benefit the beef industry. In this study we examined if muscle samples obtained from Senepol bulls have higher MFI when compared to Charolais. In addition, we determined if there was an association between single nucleotide polymorphism (SNP) in the Calpastatin gene (a cytosine insertion/deletion identified at intron 2 of the bovine Calpastatin locus (allele A = cytosine deletion; allele B = cytosine insertion) and Myofibril Fragmentation Index (MFI) of muscle samples obtained from the same animals.

Charolais × Senepol Crossbred Bulls

MATERIALS AND METHODS

LOCATION AND ANIMALS USED

Twenty-four crossbred (Charolais x Senepol) weaned calves of 9 months of age, born between February and April 2006 (n = 24) were used in this study. Every 7 days animals were rotated in two pastures (7.29 hectares e.a.) of an association of *Cynodon nlemfuensis Vanderyst var. nlemfuensis* and *Braquiaria purpurascens*. in the mountainous region, of Puerto Rico, 200 meters above sea level, with an average temperature of 20°C to 24°C and an average precipitation of 1,524 to 2,034 mm per year. The pastures were fertilized at a rate of 73.38 kg/ha/year, with a formula of 15-5-10 (N-P-K) divided into two applications (May and November).

IDENTIFICATION OF SINGLE NUCLEOTIDE POLYMORPHISMS IN THE CALPASTATIN GENE

For DNA isolation, blood samples from each animal were collected by coccigeal venipuncture. The desire CAST fragment was amplified using the polymerase chain reaction (PCR). The PCR product was visualized by electrophoresis on 1% agarose gels, stained with ethidium bromide and photographed using the Quality One ® V 4.1 software (Bio-Rad Laboratories, Richmond CA, USA) in an imaging system Gel Doc 2000 (Bio-Rad Laboratories). Either, nucleotide sequencing or single stranded conformational polymorphism (SSCP) was used to identify the correspondent CAST-SNP genotypes.

MUSCLE COLLECTION

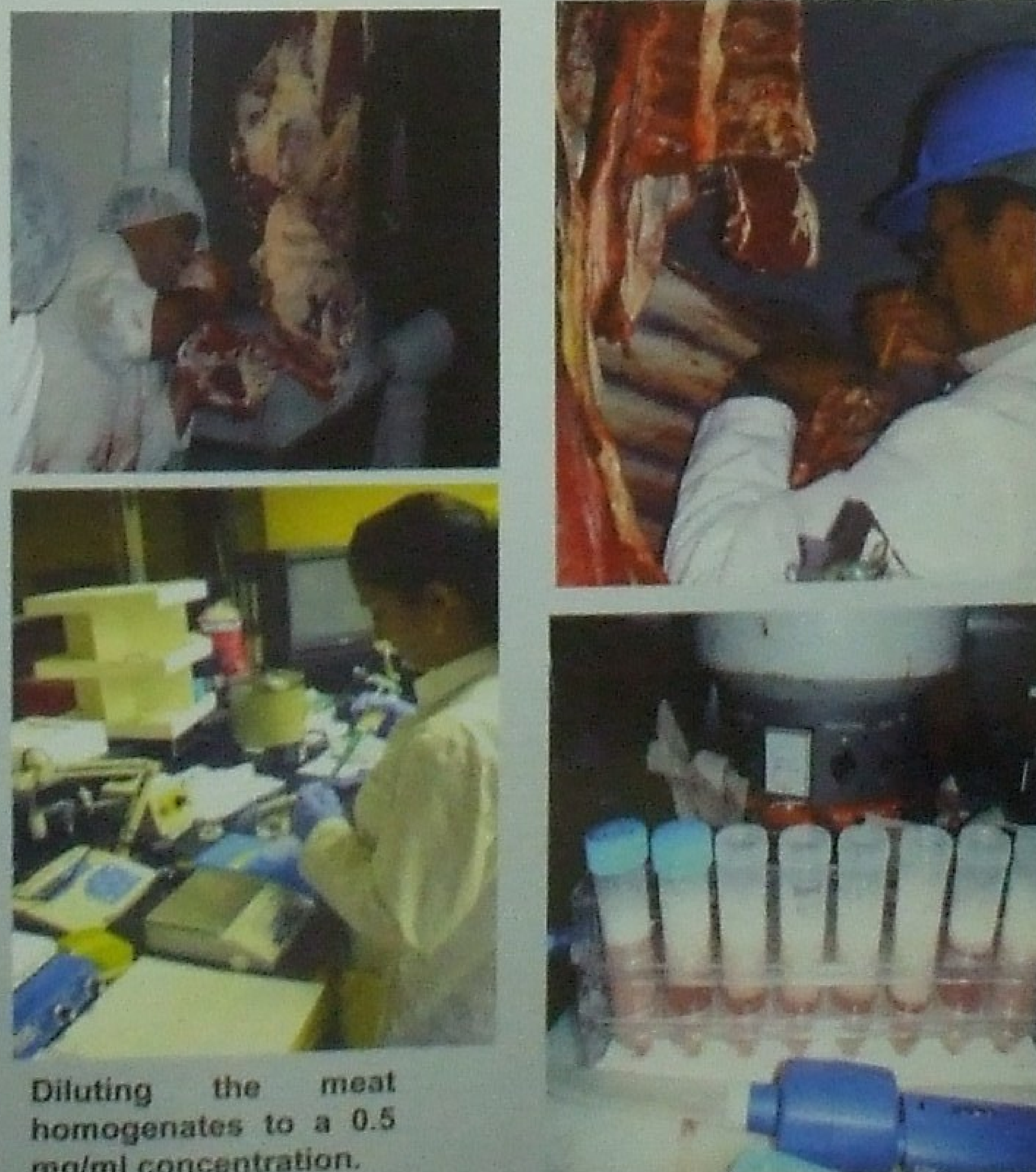
The animals were slaughtered in November 17 and December 2 of 2006 (n = 24) at an average live weight of 487 kg and an average chronological age of 20 months. From each animal were sampled three muscles: longissimus dorsi, semimembranosus and semitendinosus from the left hindquarter. The samples were aged for 14 days at 4°C and then frozen until performing the analysis.

MFI ANALYSIS

A total of 24 samples were analyzed in duplicate. The samples were thawed and homogenized using a Polytron homogenizer (30,000 RPM) in cold (4°C) homogenizing buffer (100 mM KCl; 7 mM KH_2PO_4 ; 18 mM K_2HPO_4; 1 mM EDTA; 1 mM NaN_3 [pH to 7.0]). Muscle homogenates protein concentrations were determined with the Biuret reagent assay. All muscle homogenates were diluted to a constant concentration of 0.5 mg of protein /mL of homogenization buffer. Subsequently, the absorbances of each muscle homogenate were determined at 540 nm. Absorbance results were multiplied by 200 to determine MFI values.

STATISTICAL ANALYSIS

Data were analyzed using Mixed Model Analysis of SAS (SAS, Cary, North Carolina, USA). Each animal was the experimental unit. Means were calculated considering the fixed effects of Breed or CAST-SNP; Muscle, and their interactions, while Bull, Muscle (Bull), and Repetition (Muscle) were considered random effects. When simple effects were significant (P<0.05), mean differences were analyzed using Tukey's multiple comparison test.



Diluting the meat homogenates to a 0.5 mg/ml concentration.

Homogenized meat samples in buffer (100 mM KCl; 7 mM KH_2PO_4 ; 18 mM K_2HPO_4; 1 mM EDTA; 1 mM NaN_3 [pH to 7.0]).

CONCLUSION

The longimus dorsi and semitendinosus muscle presented higher MFI than the semimembranosus. However, similar tendencies were observed within muscle between the CAST-SNP and breed effects. Therefore, it appears that animals of AA genotype and Senepol inheritance could produce more tender beef.

RESULTS

Figure 1: There was no interaction between breed and muscle (P=0.9603). Moreover, MFI values were not affected by breed (P=0.1366). The Senepol crossbred bulls showed MFI values of 45.4 (s.e. ± 4.8) for the longissimus dorsi; 24.25 (s.e. ± 2.59) for the semimembranosus; and 36.26 (s.e. ± 4.24) for the semitendinosus muscle. Charolais crossbreds showed MFI values of 33.65 (s.e. ± 2.57) for the longissimus dorsi; 20.37 (s.e. ± 2.17) for the semimembranosus; and 30.03 (s.e. ± 2.54) for the semitendinosus muscle.

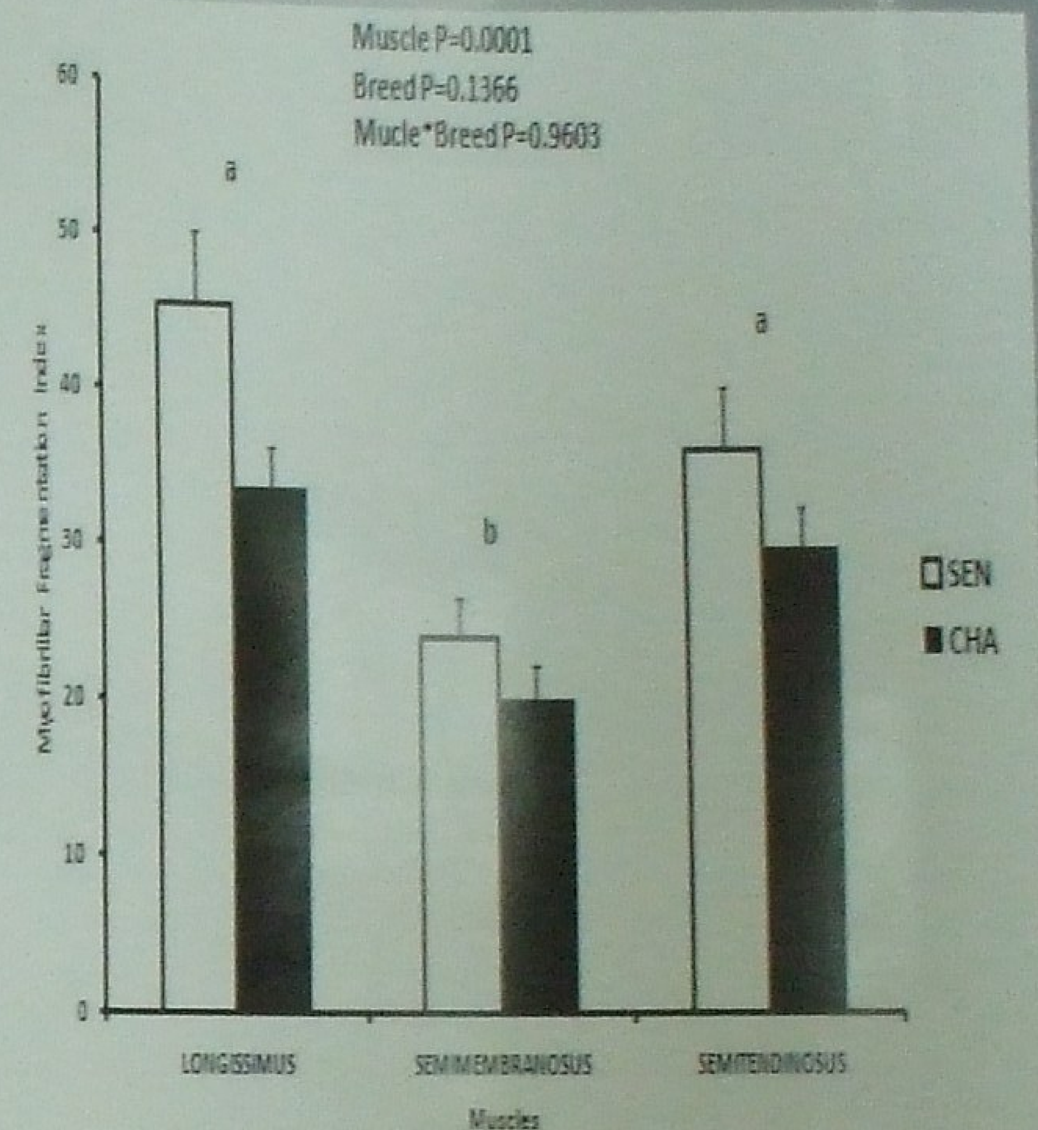
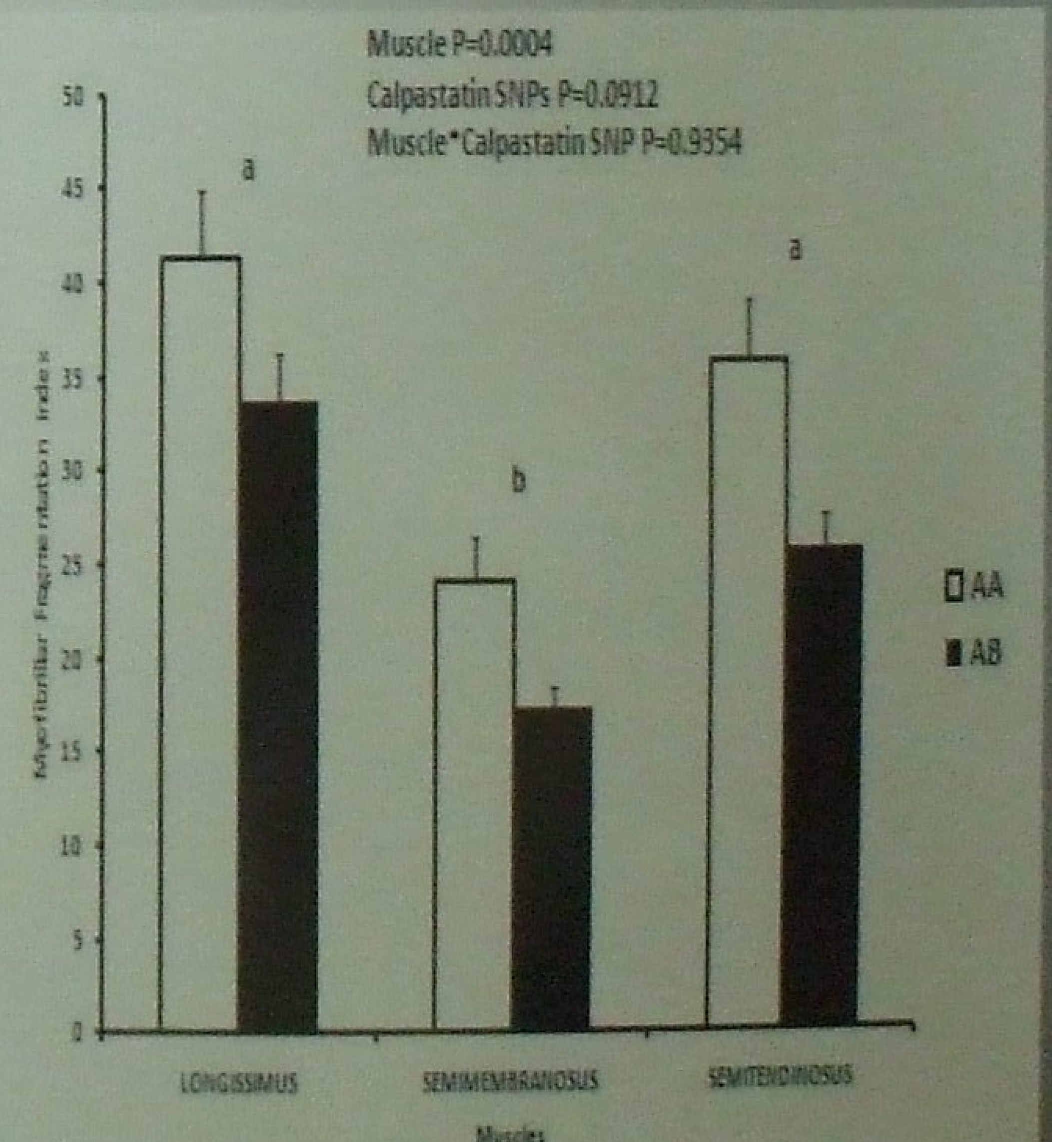


Figure 2. There was no interaction between muscle and Calpastatin-SNP (P=0.9354). However, Calpastatin-SNP tended to have an effect in MFI values. Specifically, bulls with the AA genotype tended to have higher MFI values than AB genotype (P=0.0912). Bulls with the AA genotype showed MFI values of 41.22 (s.e. ± 3.61) for the longissimus muscle; 24.17 (s.e. ± 2.22) for the semimembranosus muscle; and 35.86 (s.e. ± 3.24) for the semitendinosus muscle. Bulls with the AB genotype showed MFI values of 33.65 (s.e. ± 2.57) for the longissimus dorsi; 20.37 (s.e. ± 2.17) for the semimembranosus; and 35.86 (s.e. ± 3.24) for the semitendinosus muscle.



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