Notas Científicas

Alternative genotyping method for the single nucleotide polymorphism A2959G (AF159246) of the bovine *CAST* gene

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Abstract – The objective of this work was to genotype the single nucleotide polymorphism (SNP) A2959G (AF159246) of bovine *CAST* gene by PCR–RFLP technique, and to report its use for the first time. For this, 147 *Bos indicus* and *Bos taurus* x *Bos indicus* animals were genotyped. The accuracy of the method was confirmed through the direct sequencing of PCR products of nine individuals. The lowest frequency of the meat tenderness favorable allele (*A*) in *Bos indicus* was confirmed. The use of PCR–RFLP for the genotyping of the bovine *CAST* gene SNP was shown to be robust and inexpensive, which will greatly facilitate its analysis by laboratories with basic structure.

Index terms: calpastatin gene, meat texture, PCR–RFLP, SNP.

Método alternativo de genotipagem do polimorfismo de nucleotídeo único A2959G (AF159246) do gene *CAST* bovino

Resumo – O objetivo deste trabalho foi genotipar o polimorfismo de nucleotídeo único ('single nucleotide polymorphism – SNP) A2959G (AF159246) do gene *CAST* bovino, pela técnica de PCR–RFLP, e reportar a sua utilização pela primeira vez. Para tanto, 147 animais *Bos indicus* e *Bos taurus* x *Bos indicus* foram genotipados. A acurácia do método foi confirmada por meio do seqüenciamento direto de produtos de PCR de nove indivíduos. A menor freqüência do alelo *A*, favorável à maciez da carne, foi confirmada nos animais *Bos indicus*. O uso da PCR–RFLP, para a genotipagem do SNP do gene *CAST* bovino, mostrou-se consistente e de baixo custo, o que permite a sua análise por laboratórios dotados de estrutura básica.

Termos para indexação: gene da calpastatina, textura da carne, PCR–RFLP, SNP.

Meat tenderness is a trait strictly related to product acceptability by consumers and, thus, the genetic selection has been used for its improvement. The calpastatin gene (*CAST*) is located on bovine chromosome 7 (Bishop et al., 1993) and is a strong functional candidate for meat tenderness (Barendse, 2003). In 2001, genetic alteration in the bovine *CAST* gene (intron 6) was identified by Chung et al. (2001a) for the first time and, later, the results indicated that the use of its genotyping would allow the identification of animals with favorable enzymatic activity of calpastatin and carcass traits (Chung et al., 2001b). Posteriorly, a single nucleotide polymorphism (SNP) – characterized by the transition of an adenine by a guanine in the 3' UTR of the calpastatin gene (*CAST* SNP, A2959G – AF159246) – was identified and patented by Barendse (2003). This polymorphism has been genotyped using mass spectrometry and significant associations of it with traits related to meat quality in beef cattle were found (Casas et al., 2006; Morris et al., 2006). However, analysis of the restriction map of the sequence that contains this polymorphism showed that it can be genotyped by polymerase chain reaction restriction fragment length polymorphism (PCR–RFLP), since it is located at a *Dde* I recognition site.

The objective of this work was to genotype the SNP A2959G (AF159246) of bovine *CAST* gene by PCR–RFLP technique, and to report its use, for this purpose, for the first time.

One-hundred forty-seven bovine, of which 46 were pure Nellore (*Bos indicus*), 41 Canchim (5/8 *Bos taurus* + 3/8 *Bos indicus*), 26 Rubia Gallega x Nellore crossbred (1/2 *Bos taurus* + 1/2 *Bos indicus*), 19 Brangus three-way cross (9/16 *Bos taurus* + 7/16 *Bos indicus*), and 15 Brown Swiss three-way cross (3/4 *Bos taurus* + 1/4 *Bos indicus*), were genotyped using forward 5' AAT ATA TGC GCT TCC TGG TCT GTC CAG 3' and reverse 5' AAT ATA TTC TCC CCA CAG TGC CTG TAA 3' primers (Morris et al., 2006), along with *Dde* I restriction endonuclease.

The amplification reactions were performed in a final volume of 25 µL containing 50 ng of DNA, 0.2 µM of each primer, 1x PCR buffer (20 mM Tris-HCl, pH 8.4 and 50 mM KCl), 1.5 mM $MgCl_2$, 0.24 mM of each dNTP, and 0.75 U T*aq* DNA polymerase. After initial denaturation at 95ºC for 5 min, amplification was performed in 38 cycles at 95ºC for 1 min, 54ºC for 1 min, and 72ºC for 2 min, followed by a final extension step at 72ºC for 5 min. Twelve microliter aliquots of the amplification products were digested with 3 U of *Dde* I at 37°C for 4 hours. DNA fragments were separated on 2% agarose gel for 90 min and were visualized with bromide staining and exposure to ultraviolet light.

Based on the genotypes identified on gels, allele frequencies were calculated according to Weir (1996). Differences in allele frequencies of the polymorphism between genetic groups were determined by qui-square test (χ^2) of SAS software (SAS Institute, 1999).

Amplified products of 269 bp were obtained which, when submitted to digestion, revealed the *A* (137 and 132 bp) and *G* allele (269 bp) (Figure 1). The accuracy of bovine *CAST* gene SNP A2959G (AF159246) genotyping by PCR–RFLP was confirmed through the direct sequencing of PCR products of nine individuals. DNA amplification failure was not observed in the studied genetic groups. The absence of null alleles with the used primers was important, since many markers described in the literature cannot be genotyped in certain populations, due to no amplification, which results in a problem for the marker assisted selection.

The *A* allele frequencies found in the studied genetic groups – Nellore, Canchim, Rubia Gallega x Nellore, Brangus three-way cross, and Brown Swiss three-way cross – were 0.42, 0.70, 0.85, 0.84, and 0.73, respectively. Despite the small number of individuals of some genetic groups, a significant increase ($p<0.01$) of the *A* allele frequency was observed in *Bos taurus* x *Bos indicus* animals. These results agree with Casas et al. (2006) who, despite the higher *A* allele frequency observed in *Bos indicus* Brahman (0.72), also observed the highest *A* allele frequency in animals *Bos taurus* (0.80) and *Bos taurus* x *Bos indicus* (0.83). Working exclusively with populations of *Bos taurus* breeds, Morris et al. (2006) found frequencies of the *A* allele between 0.84 and 0.99. The frequency of the *A* allele, favorable for the meat traits (Barendse, 2003), lower in *Bos indicus* than in *Bos taurus* x *Bos indicus* animals was expected, since, according to Wheeler et al. (1994), the *Bos indicus* breeds produce less tender meat, when compared to *Bos taurus* and *Bos taurus* x *Bos indicus* animals.

In conclusion, the use of the PCR–RFLP technique, for the genotyping of A2959G (AF159246) SNP of the bovine *CAST* gene, was shown to be robust and

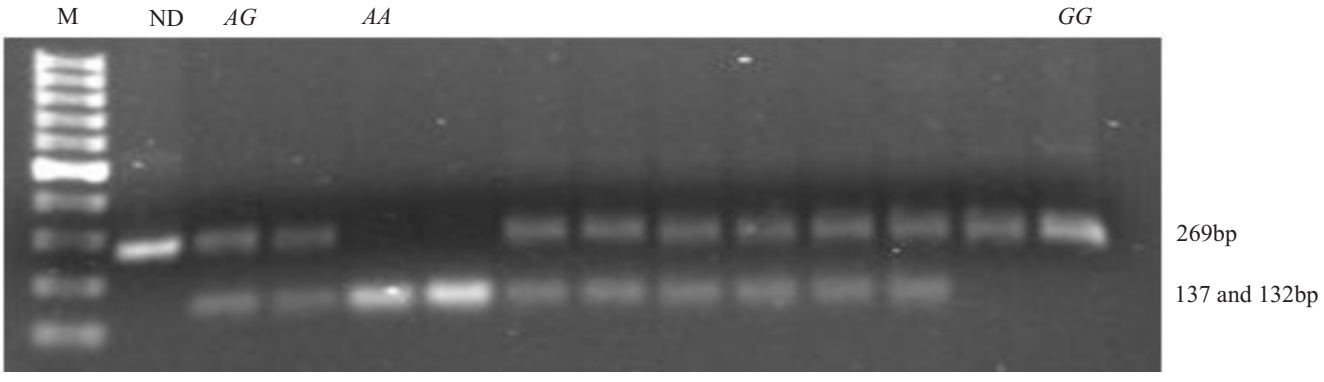

Figure 1. Bovine *CAST* gene SNP A2959G (AF159246) genotyping by PCR–RFLP, using *Dde* I endonuclease. M: 100 bp molecular weight standard; ND: undigested product of 269 bp; *AA*: genotype characterized by the presence of 137 and 132 bp fragments; *AG*: heterozygous genotype characterized by 269, 137 and 132 bp fragments; *GG*: genotype characterized by 269 bp fragments. The 137 and 132 bp fragments presented as superimposed under electrophoresis conditions performed.

inexpensive, which will greatly facilitate analysis of this polymorphism through basic laboratory equipment and reagents, when compared to the mass spectrometry method.

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