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# Analysis of a polymorphism in the DGAT1 gene in 14 cattle breeds through PCR-SSCP methods

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**Abstract**

The diacylglycerol *O*-acyltransferase (DGAT1) is a microsomal enzyme that catalyzes the final step of triglyceride synthesis. Recent work have evidenced a significant association between lysine at amino acid position 232 with elevated milk fat content, while an alanine at this position is associated with lowered milk fat content. The aim of the present work was to develop a simple and inexpensive PCR-SSCP assay in order to discriminate the CG/AA alleles in exon 8 of the DGAT1 gene. In addition, this method was used to analyze the polymorphism of the DGAT1 through PCR-SSCP methods in 14 populations of cattle from Argentine, Bolivia and Uruguay. The PCR primers were designed from GenBank reported sequences. In this study, we found three PCR-SSCP variants, which were denominated from "A" to "C". However, DNA sequencing analysis showed that "A" variant corresponded with the A allele, while both "B" and "C" observed pattern have the motif AA at positions 10,433–10,434 (K allele), being two alternative conformations of the same DNA sequence. Both variants were detected within each breed with the exception of Hereford, and the heterozygosity varied between 0.000 and 0.524. The gene frequency analysis evidenced significant differences among the studied breeds ($F_{ST} = 0.325$, $p = 0.000$). European *Bos taurus* breeds, with the exception of Jersey breed, showed the lowest frequency of the K allele, while highest K allele frequencies were harboured by *Bos indicus* type cattle. In addition, unselected South American Creole cattle breeds and the synthetic Brangus breed had intermediate allele frequencies.


*Keywords:* Cattle; Creole breeds; DGAT1; PCR-SSCP

## 1. Introduction

The presence of a quantitative trait loci (QTL) in the centromeric end of chromosome 14 with a major effects on milk fat content in dairy cattle, has been supported by many studies (Coppieters et al., 1998; Heyen et al., 1999; Riquet et al., 1999; Boichard et al., 2000; Looft et al., 2001). Grisart et al. (2001) reported a strong positional candidate gene for milk fat content in a 3cM interval the diacylglycerol *O*-acyltransferase (DGAT1).

A lysine/alanine (K232A) substitution on the protein encoded by the bovine DGAT1 gene (EC 2.3.1.20) has been shown to be associated with milk fat content in different breeds, such as Holstein-Friesian, Fleckvieh and Jersey (Grisart et al., 2001; Spelman et al., 2002; Winter et al., 2002). In cattle, the lysine variant of DGAT1 is associated with elevated milk fat content, while an alanine at this position is associated with lowered milk fat content. It has been hypothesized that a lysine residue at position 232 of the DGAT1 protein, as it is found in all non-bovine mammalian species studied so far, could confer more efficient binding of acyl-coenzyme A than an alanine residue at this position (Winter et al., 2002), being probably the most likely cause of the BTA14

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QTL effect. Recently, Grisart et al. (2004) provided strong evidence that support the causality of the K232A DGAT1 polymorphism in the determinism of the proximal BTA14 QTL on milk yield and composition. In addition, Thaller et al. (2003) showed that the lysine allele of DGAT1 has also a positive effect on intramuscular fat content in the Charolais and Holstein breeds.

Detection of allelic variation at positions 10,433–10,434 of the DGAT1 gene in different breeds has been performed by diverse assays, such as PCR-RFLP, oligonucleotide ligation assay (OLA), and DNA sequencing (Grisart et al., 2001; Winter et al., 2002; Kaupe et al., 2004). The purpose of the current study was to develop a simple and unexpensive PCR-SSCP assay in order to discriminate the CG/AA alleles in exon 8 of the DGAT1 gene. Such a test could be useful in the genetic characterization of different cattle populations, including studies in the area of population genetics and association analyses with milk production traits.

## 2. Materials and methods

### 2.1. Sample collection

Blood samples were collected from 144 unrelated South America Creole cattle from Argentina and Bolivia belonging to the following breeds: Argentine Creole, Saavedreño Creole, Creole of Valle Grande, Chaqueño Boliviano Creole, and Chusco Creole. Also, 118 unrelated animals from European commercial breeds (Hereford, Jersey, Aberdeen Angus, Holstein, Charolais and Normande), 26 bovines from indicine cattle breeds (Nelore and Brahman), and 8 individuals from the synthetic Brangus breed (Br) were sampled.

### 2.2. DNA extraction and PCR-SSCP DGAT1 analysis

Total DNA was extracted from blood samples using the DNAzol purification kit (Invitrogen, Carlsbad, CA, USA), following manufacturer instructions. PCR primers (F 5′-CTTGCTCGTAGCTTTGGCAGG-3′ and R 5′-CGAAGAGGAAGTAGTAGAGATC-3′) were designed from reported sequences (Genbank accession numbers AY065621 and AJ318490, Grisart et al., 2001 and Winter et al., 2002) in order to amplify a 176 bp fragment of exon 8 of the DGAT1 gene spanning the K232A substitution. The 25 μl reaction mix contained 2 μl of total DNA, 0.4 μM of each primer, 0.1 mM of dNTPs and 0.8 U of *Taq* polymerase (Invitrogen) in 20 mM Tris–HCl (pH 8.4), 50 mM KCl and 4 mM $MgCl_2$, under mineral oil. The PCR consisted in 30 cycles of 45 s at 94 °C, 45 s at 59 °C and 45 s at 72 °C, with a final elongation step of 7 min at 72 °C. Ten microliters of each PCR product was added to 16 μl of loading dye (96% formamide, 0.01 M EDTA pH 8, 0.05% bromophenol blue, 0.05% xylene cyanol) and 10 μl of water. The samples were then heated at 96 °C for 10 min, cooled on ice for at least 5 min and loaded onto a 10% polyacrylamide gel (38:1 acrylamide: bisacrylamide). Electrophoresis was carried out at 4 °C, 3200 V/h in 0.5 × TBE buffer. The gels were subsequently fixed in 5% ethanol, stained with 0.2% $AgNO_3$ and revealed with 2% NaOH. DNA with known genotypes (AA, AK and KK) for the K232A substitution, were use as positive controls.

### 2.3. DNA sequencing

The PCR products from 8 individuals which presented different PCR-SSCP patterns, including both homozygotes and heterozygotes genotypes were cloned into TOPO TA Cloning kit, according to the manufacturer's instructions (Invitrogen). Several inserts from positive clones for each animal were confirmed by PCR using the primers mentioned above. SSCP patterns were determined in each positive clone and clones corresponding to different PCR-SSCP band patterns were chosen for DNA sequencing. Three clones of each distinct SSCP pattern, that correspond to different clones and individuals, were sequenced in both directions on an Applied Biosystems 377 automated sequencer (Bio-Resource Center, Cornell University, Ithaca, NY, USA), using the T7 universal primer. Sequence was accepted if at least four of six reactions produced identical results at a given base.

### 2.4. Data analysis

Sequences were aligned using CLUSTAL-W version 1.7 (Baylor College of Medicine, Human Genome Sequencing Center, Houston, TX, USA; Thompson et al., 1994). Gene frequencies were determined for each breed by direct counting. Levels of genetic variability were estimated with the number of alleles ($n_a$) and the unbiased expected heterozygosity ($h_e$), computed according to Nei (1987). $F_{ST}$ distances were calculated using the Arlequin software (Schneider et al., 2000). Exclusion power was calculated according to Weir (1996).

## 3. Results and discussion

Three PCR-SSCP variants were identified, which were at first designated "A" to "C" (Fig. 1). Variants "A" and "B" had the same pattern as control samples corresponding to A and K alleles previously reported by Grisart et al. (2001), respectively. Sequence alignment showed that the "A" and "B" SSCP variants corresponded to haplotypes GC and AA at positions 10,433 and 10,434, respectively. The analysis also evidenced that DNA sequences corresponding to patterns "B"