SINGLE-STRAND CONFORMATION POLYMORPHISM (SSCP) ANALYSIS OF αS_1-CASEIN, β-CASEIN AND κ-CASEIN GENES IN CHARNEQUEIRA PORTUGUESE INDIGENOUS GOAT BREED

ANÁLISIS POR POLIMORFISMO CONFORMACIONAL DE CADENA MONOCATENARIA (SSCP) DE LOS GENES αS_1-CASEÍNA, β-CASEÍNA Y κ-CASEÍNA EN LA CABRA PORTUGUESA AUTÓCTONA CHARNEQUEIRA

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PALABRAS CLAVE ADICIONALES

Cabras. SSCP. PCR. Polimorfismos genéticos. αs_1-caseína. β-caseína. κ-caseína.

ADDITIONAL KEYWORDS

Goat. SSCP. PCR. Genetic polymorphisms. αs_1-casein. β-casein. κ-casein.

SUMMARY

The DNA from eighty goats belonging to the indigenous portuguese caprine breed Charnequeira, ecotype from Beira Baixa (Beiroa), was analysed. Single-strand conformation polymorphisms were identified at exon 9 and at exons 10-11 of the αs_1-casein gene and at exon 4 of the κ-casein. The β-casein gene was found monomorphic at exon 7 in this population sample. The establishment of an association of some of these SSCP polymorphisms with milk production and protein and fat content was attempted.

RESUMEN

Se analizó el DNA de ochenta cabras autóctonas portuguesas de la raza Charnequeira, ecotipo de la Beira Baixa (Beiroa). Se identificaron polimorfismos conformacionales de cadena monocatenaria en el exón 9 y en los exones 10-11 del gen de la αs_1-caseína y en el exón 4 de la κ-caseína. El gen de la β-caseína se identificó como monomórfico en el exón 7 en el muestreo de esta población. Se intentó el establecimiento de relaciones entre algunos de estos polimorfismos de SSCP y la producción de leche, contenido proteico y de grasa.

INTRODUCTION

Single-strand conformation polymorphism (SSCP) is a powerful method for identifying sequence variation in amplified DNA. SSCP analysis of DNA have been used for detection of genetic mutations in humans (Orita *et al.,* 1989), rats (Pravenec *et al.,* 1992), cattle (Kirkpatrick, 1992) and in various bacteriological (Morohoshi *et al.,*

1991) and viral (Fujita *et al.,* 1992) systems.

The search for SSCP polymorphisms could lead to the finding of genetic markers useful for improved selection of agricultural populations, namely when applied to candidate genes associated with quantitative genetic variation in traits of economic importance.

Most significant studies using the SSCP approach were accomplished on bovines in linkage analysis (Neibergs *et al.,* 1993) and to define intragenic haplotypes at the growth hormone (Lagziel *et al.,* 1996).

The present study is part of a genetic improvement programme of the indigenous portuguese caprine breed Charnequeira. Charnequeira goats are reared mainly in the northern part of Alentejo, southern part of Beira Baixa (Castelo Branco, Idanha-a-Nova, Penamacor, Vila Velha do Ródão) and also in southern Alentejo (Sines, Santiago de Cacém, Odemira) where they are very well adapted to their natural environment. They are used for meat and for cheese. This work describes some SSCP polymorphisms at the αs_1-casein, β-casein and κ-casein genes from 80 goats belonging to the indigenous portuguese caprine breed Charnequeira, ecotype from Beira Baixa (Beiroa).

MATERIAL AND METHODS

DNA SAMPLES

DNA was obtained from peripheral blood leukocytes of 80 animals of Charnequeira goat breed ecotype Beiroa using a DNA isolation kit from Puregene.

PCR AMPLIFICATION

The lactoprotein genes were amplified by PCR using the four primer pairs shown in **table I**. Four amplification fragments were generated ranging in size from 257 to 500 bp. PCR amplification was carried out for each primer pair using Ready-To-Go PCR Beads (Amersham Pharmacia Biotech) according to the following conditions. Each lyophilized Bead contains 1.5 units of *Taq* DNA polymerase, 10 mM Tris-HCl (pH 9), 50 mM KCl, 1.5 mM $MgCl_2$ and 200 μM of each dNTP. The reaction mixture was completed with 16 pmol of each primer (32 pmol for amplification of exon 4 of κ-casein) and 50 ng of genomic DNA. For amplification of exon 7 of β-casein a final concentration of 2.5 mM of MgCl2 was used. The amplification began with denaturation at 95ºC for 5 min followed by 30 cycles of denaturation at 95ºC for 30 s, annealing at 55ºC, 57ºC or 60ºC during 30s and extension for 30 s at 72ºC and was concluded with a final extension at 72ºC for 5 min.

SSCP ANALYSIS OF THE αs_1-CASEIN, β-CASEIN AND κ-CASEIN GENES

For SSCP analysis, 5 (l of each amplification product were added to 15 μl of stop solution (95 p.100 formamide, 10 mM NaOH, 0.05 p.100 xylene cyanol and 0.05 p.100 bromophenol blue). The samples were heat-denatured at 95ºC for 5 min and chilled on ice, and loaded onto a 9 p.100 (exon 9 of αs_1-casein), 12 p.100 (exons 10-11 of αs_1-casein), 7 p.100 (exon 4 of κ-casein) or 8 p.100 (exon 7 of β-casein) polyacrylamide/TBE 0,5x gel containing, only for exon 4 of κ-casein and exon 7 of β-

casein, 5 p.100 of glycerol. Gels were run at 25W (αs_1-casein) or 30 W (κ-casein and β-casein) for 2 h 50 min (exon 9 of αs_1-casein), 3 h 30 min (exons 10-11 of αs_1-casein), 4 h 30 min (exon 4 of κ-casein) or 5 h 30 min (exon 7 of β-casein) at 15ºC while cooling using a DCode™ Universal Mutation Detection System (BIO-RAD). After the run the gel was removed from the apparatus and the DNA bands were visualised through silver staining.

RESULTS

SSCP POLYMORPHISMS

We have chosen the exon 7 of the β-casein gene, the exon 4 of the κ-casein gene and the exons 9 and 10-11 of the αs_1-casein gene for the SSCP analysis.

The analysis of the amplified fragments by the PCR method using the primers described in **table I** is shown in **figure 1**. Their lengths correspond to those expected according to the position of the primers deduced from the described nucleotide sequence of the corresponding genes from *Capra hircus* (Vilotte *et al.,* 1991; Leroux *et al.,* 1992; Roberts *et al.,* 1992). Considering the eighty Charnequeira goats the following SSCP polymorphisms were found inside each of those fragments (**figures 2, 3, 4** and **table II**). Five conformational patterns were found in exon 9 of the αs_1-casein gene and three conformational patterns in exons 10-11 of the same gene. Two conformational patterns were identified in exon 4 of the κ-casein gene. The exon 7 of the β-casein gene was found monomorphic (not shown).

Table I. *SSCP fragments at the casein genes and PCR analysis parameters for the Charnequeira goat breed..* (Fragmentos de SSCP de genes de las caseinas y parámetros de análisis PCR para la cabra de la raza Charnequeira).

Gene	Composition[1]	Length (bp)	Primer sequences 5'→3'[2]	T[3] (°C)
αs_1-Cn	I8, E9, I9	266[4]	F - GTTAGCAACCCATTAAGTGTGG R - GGATAGAGCTACATACATAGT	55
αs_1-Cn	I9, E10, I10, E11, I11	315[4]	F - TGATGTGTCTGGTTAATTAGC R - CACAACATTCTTGCTCATTCC	60
β-Cn	I6, E7, I7	510[5]	F - CTTCTTTCCAGGATGAACTCC R - GACTTACAAGAATAGGGAAGG	60
κ-Cn	E4	416[6]	F - GAGAAAGATGAAAGATTCTTCG R - GCTTCTGGATTATCTACAGTG	57

[1] Fragment composition: E1, E2, …exon 1, exon 2,…; I1, I2, …, intron 1, intron 2,….

[2] F: forward primer; R: reverse primer.

[3] Optimum annealing temperature.

[4] Leroux *et al.,* 1992.

[5] Roberts *et al.,* 1992.

[6] Chikuni *et al.,* 1995.

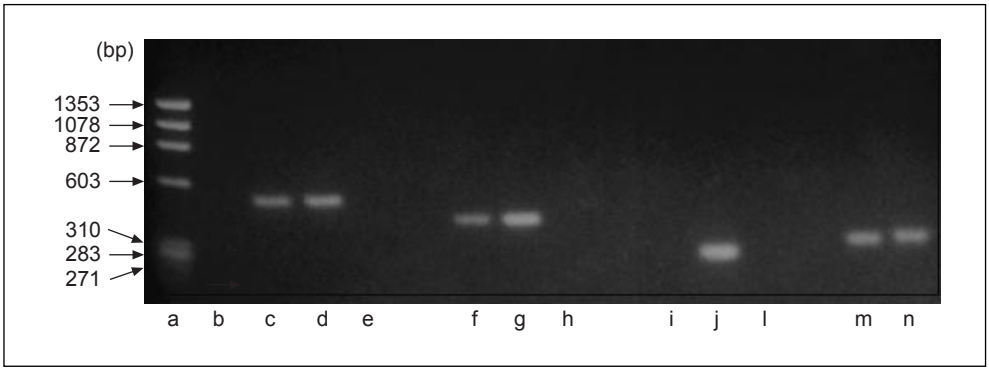

Figure 1. *2% Agarose gel electrophoresis of the fragments amplified with the primers specific to β-casein exon 7 (c and d), κ-casein exon 4 (f and g), αs$_1$-casein exon 9 (j) and αs$_1$-casein exons 10-11 (m and n); b, e, h, i and l are negative controls; a - molecular marker (*Hae*III f x174 (in bp).* (Electrophoresis en gel de agarose a 2% de fragmentos amplificados con primers específicos para la β-caseina exón 7 (c y d), κ-caseina exón 4 (f y g), αs$_1$-caseina exón 9 (j) y αs$_1$-caseina exones 10-11 (m y n); b, e, h, i y l son controles negativos; a - marcador molecular (*Hae*III f x174 (en bp)).

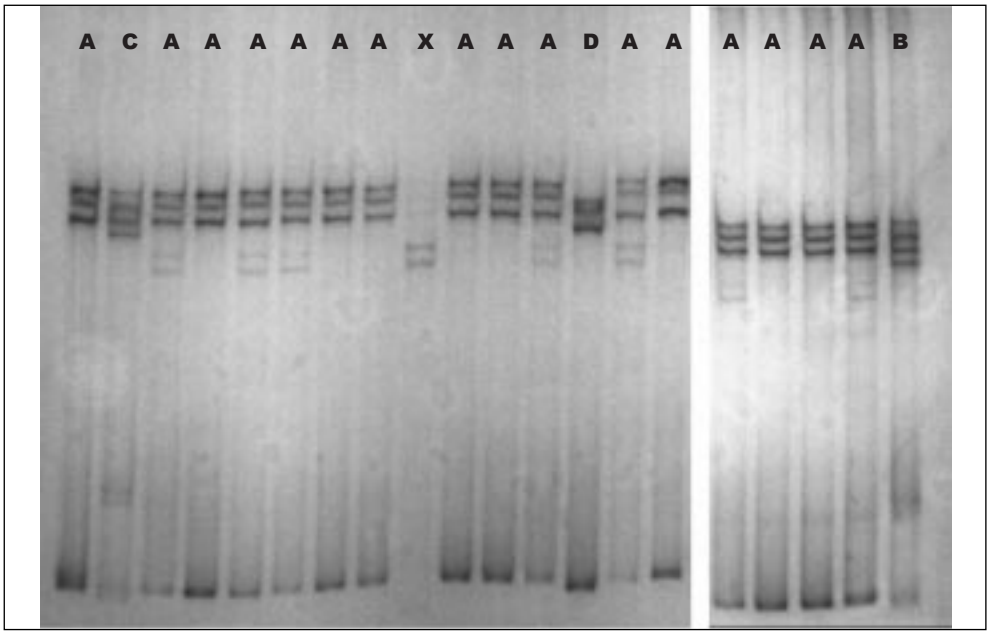

Figure 2. *SSCP analysis at exon 9 of the αs$_1$-casein gene in Charnequeira goat breed (20 animals). A to D - conformational patterns; X - no amplification.* (Análisis SSCP del exon 9 del gen de la αs$_1$-caseina en la cabra Charnequeira (20 animales). A a D - padrones conformacionales; X - sin amplificación).

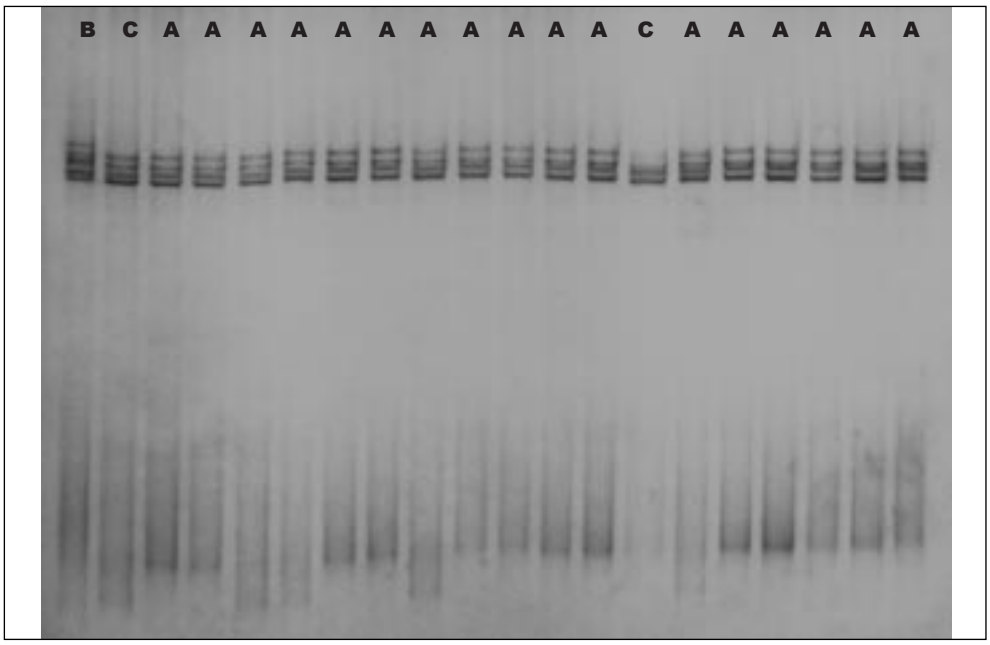

Figure 3. *SSCP analysis at exons 10-11 of the αs_1-casein gene in Charnequeira goat breed (20 animals). A to C - conformational patterns.* (Análisis SSCP de los exones 10-11 del gen de la αs_1-caseina en la cabra Charnequeira (20 animales). A a C - padrones conformacionales).

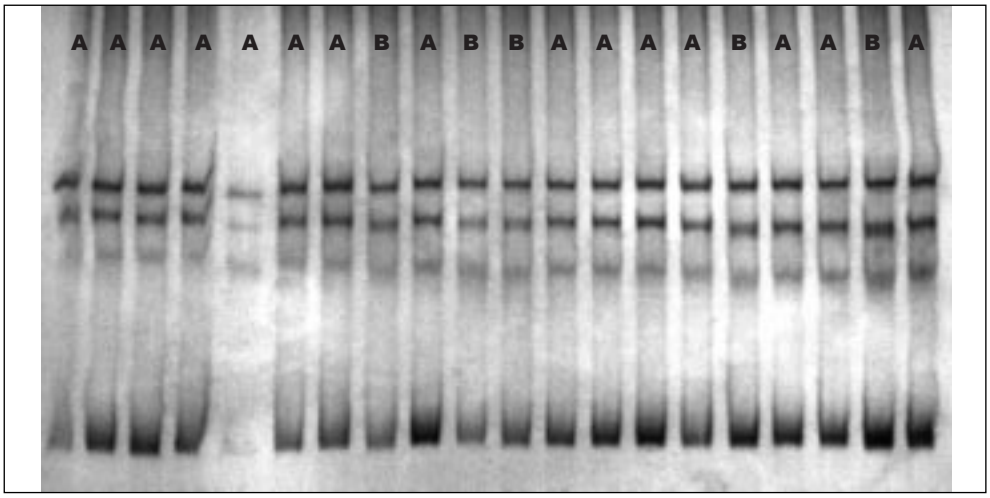

Figure 4. *SSCP analysis at exon 4 of the κ-casein gene in Charnequeira goat breed (20 animals). A and B - conformational patterns.* (Análisis SSCP del exón 4 del gen de la κ-caseina en la cabra Charnequeira (20 animales). A y B - padrones conformacionales).

Table II. *Schematic representation of SSCP patterns for the indicated Charnequeira goat casein exons and respective frequencies.* (Representación esquemática de los padrones SSCP para los exones de las caseinas de cabra Charnequeira indicados y respectivas frecuencias).

	Pattern A	Pattern B	Pattern C	Pattern D
αs_1-Cn Exon 9	 87,5%	 1,25%	 6,25%	 3,75%
αs_1-Cn Exon 10-11	 93,75%	 1,25%	 5%	
κ-Cn Exon 4	 77,5%	 22,5%		
β-Cn Exon 7	 100%			

SEARCH FOR AN ASSOCIATION OF SSCP POLYMORPHISMS WITH MILK PRODUCTION AND WITH PROTEIN AND FAT CONTENT

We have made a preliminary attempt towards the search for associations between the SSCP polymorphisms and quantitative variation in the productive traits. However, the results obtained using the SAS programme applied to the analysis of linear contrasts of SSCPs relating to milk production, protein and fat content for each exon analysed did not allow to conclude to the existence of any association between the observed polymorphisms and productive trait in this sample.

DISCUSSION

Milk composition, namely its protein content, is the main factor influencing the yield of cheese production. The polymorphism of milk proteins affect the milk composition and cheese quality. Caseins, in particular, have been proposed as polymorphic markers for the selection of goats in order to improve the yield and the quality of cheese. The SSCP analysis of milk protein genes could be a valuable alternative approach for establishing allelic variants useful as markers to aid selection. We have applied this technique to exon 7 of the β-casein gene, exon 4 of

the κ-casein gene and exons 9 and 10-11 of the αs_1-casein gene from the indigenous portuguese caprine breed Charnequeira ecotype Beiroa. The SSCP polymorphisms we have found in these genes coding for milk proteins hint at the possibility of exploring this approach for the search of genetic markers located in these genes. In spite of the impossibility of concluding through statistical analyses within our sample, to the existence of associations between the polymorphisms observed and milk production and protein and fat content the SSCP polymorphic variation makes these genes potential candidates for the establishment of associations with quantitative traits. Indeed, our sample is too small and our analysis limited to a few number of exons containing a small portion of the genes. At the protein level various casein polymorphisms were determined in goats and correlated with milk composition and physicochemical proprieties (Manfredi *et al,* 1993; Dall'Olio *et al,* 1989). The SSCP approach could be used in a more extended study for the establishment of a correspondence with the observed protein polymorphism. The complete analysis of the exons of casein genes should lead to the definition of haplotypes representing all the DNA sequence alleles present at these casein genes in a larger population of this breed. If it is possible to specifically define haplotypes at these candidate genes that can be associated with milk production, protein and fat content it would be rendered available a valuable genetic resource for improvement of this caprine breed.

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