GENOTYPING THE ß-CASEIN LOCUS IN CATTLE USING PCR

D. Savva, S.J. Pinder and C.J. Skidmore
Department of Biochemistry & Physiology, University of Reading,
Whiteknights, P.O. Box 228, Reading RG6 2AJ, UK

SUMMARY

We have designed a rapid test, based on the polymerase chain reaction (PCR) and allele-specific oligonucleotides to ascertain the presence of two alleles (A^3 and B) at the ß-casein locus. Blood and semen can be used as sources of DNA for the test. Survey of 108 Holstein-Friesian bulls confirms that both these alleles are rare in the population and that the B allele is tightly linked to the B allele of κ-casein.

INTRODUCTION

Polymorphisms in bovine milk proteins are well documented (Eigel *et al.*, 1984). In the case of ß-casein there are seven variants referred to as A^1, A^2; A^3, B, C, D and E, of which D and E are not found in Holstein-Friesian cattle. The A^3 variant has been associated with a significant increase in milk yield per lactation (Ng-Kwai-Hang *et al.*, 1984) without any observed detrimental effects. It might therefore be desirable for dairy farmers to increase the frequency of the A^3 allele of ß-casein in their herds. Other alleles may also be associated with useful production traits.

In order to facilitate the organisation of suitable breeding programmes, a reliable test for the alleles of ß-casein is required. We have examined the nucleotide sequence of the ß-casein cDNA (Stewart *et al.*, 1987; Jimenez-Flores *et al.*, 1987) and have devised a test for the A^3 and B alleles based on the differential hybridisation of allele-specific oligonucleotides to a 328 bp PCR fragment from the ß-casein gene (nucleotides 259-587 in the numbering of Stewart *et al.*, 1987) which contains the mutations of interest (Figure 1).

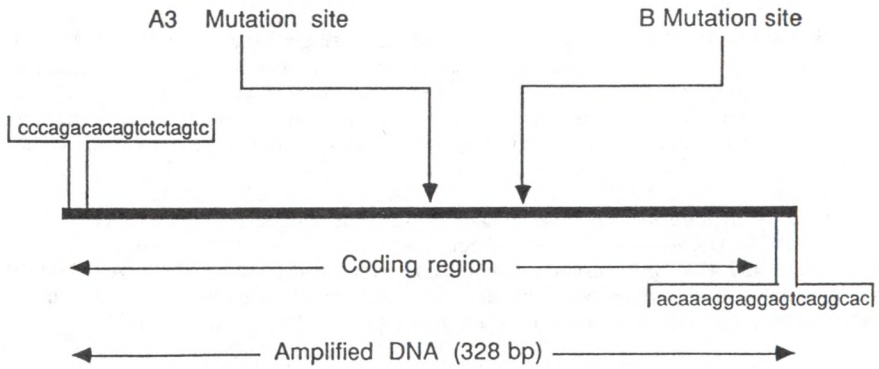

Figure 1 Diagram of the PCR test for ß-casein

MATERIAL AND METHODS

Samples: Blood and semen samples were supplied by the MAFF Cattle Breeding Centre, Shinfield (Dr. P. Lamont), the AI Organisation of the Milk Marketing Board (Dr. R. Shaw) and from cattle at the AFRC Institute for Grassland and Animal Production, Shinfield.

DNA extractions: DNA from blood was isolated essentially according to Boulnois (1987). Semen DNA was isolated by a similar method but after pretreatment overnight in a solution containing 5% (w/v) sodium dodecyl sulphate (SDS), 0.2% (w/v) proteinase K and 5% (v/v) 2-mercaptoethanol.

Amplification of DNA: PCR was used to amplify a 328 bp fragment of the ß-casein gene. Each reaction (100µl) contained 10µl of 10x reaction buffer (100mM Tris-HCl, pH 8.3, 500mM KCl, 15mM $MgCl_2$, 0.1% (w/v) gelatin), 200µM of each dNTP, 1µM of each of the two oligonucleotide primers (5'-CCCAGACACAGTCTCTAGTC-3' and 5'-CACGGACTGAGGAGGAAACA-3'), 100ng of genomic DNA and 2.5 units of Amplitaq™ DNA polymerase. After 5 min at 95°C, 30 cycles of amplification were carried out using a programmable Dri-block (PHC-1; Techne, Cambridge, UK); each cycle consisted of 1 min at 95°C, 1 min at 50°C, and 3 min at 74°C.

Detection of genotypes: Dot blots were prepared using a Hybri-dot™ filtration manifold. The membranes were washed briefly in 2xSSC and air-dried for 45 min. They were then baked at 80°C for 60 min and UV-irradiated for 30 sec. Membranes were prehybridised for 3 h in 50 ml 2xSSC containing 0.5% (w/v) dried skimmed milk powder, 1mM Na_2EDTA and 4nM unlabelled dCTP at an appropriate temperature. The allele-specific oligonucleotide probe (end-labelled using T4 polynucleotide kinase and γ-^{32}P-ATP (3000 Ci/mmol) was then added and hybridised overnight under the same conditions. The membranes were washed in 2xSSC and then in 0.2xSSC until no further loss of activity was apparent. The damp filters were wrapped in Saran-wrap and autoradiographed. Before reprobing, membranes were incubated for 30 min at 65°C in 0.2xSSC and stored wet in Saran-wrap at -20°C.

RESULTS

When PCR amplification of cattle DNA was carried out, a single band of the expected size (328 base pairs) could be seen following electrophoresis on agarose gels. Allele-specific oligonucleotides were designed with reference to the amino acid sequences of the A^3 and B variants of ß-casein. In each case a pair of oligonucleotides was prepared, one corresponding to the variant DNA sequence and the other to the published cDNA sequence (which codes for the A^2 variant). The A^3-specific oligonucleotide was 5'-CCTAAGCA(A/G)AAAGAAAT-3' and the non-A^3-specific was 5'-CCTAAGCACAAAGAAAT-3' (nucleotides 411-427): these could be discriminated by hybridisation and washing at 37°C. The B-specific oligonucleotide was 5'-TTACTGAAAG(A/G)CAGAGC-3' and the non-B-specific was 5'-TTACTGAAAGCCAGAGC-3' (nucleotides 457-473): hybridisation and washing were carried out at 43°C. A hybridisation signal of equal intensity with both specific and non-specific oligonucleotides was taken to indicate a heterozygote: homozygotes give a signal with only one of the two probes.

The ß-casein phenotypes of 133 high-yielding cows were obtained by polyacrylamide gel electrophoresis. 13 cows carried the A^3 allele and 14 the B allele: all were heterozygotes. DNA samples from these cows were used to confirm the specificity of the tests. Derived genotypes agreed completely with the known phenotypes of the animals. DNA from 108 Holstein-Friesian bulls has now been analysed. In this population there are 3 A^3 heterozygotes, 6 B heterozygotes and 1 B homozygote giving allele frequencies of 0.01 for A^3 and 0.04 for B. Grosclaude (1988)

notes that in French Holsteins, ß-casein B is always associated with κ-casein B. This is confirmed in our population, where, of 17 ß-casein B carriers tested, 15 were AB and 2 BB at the κ-casein locus. This indicates a high degree of linkage disequilibrium between the two loci.

DISCUSSION

In the absence of useful RFLPs, the PCR can be used to produce tests for genotyping the casein loci, based on the use of allele specific oligonucleotides. Such a test has been successfully developed here for two alleles of the ß-casein locus. The test is accurate and rapid. Although currently it involves the use of radioactivity, the presence of large amounts of DNA from the PCR reaction makes it easy to adapt to non-radioactive probes.

The frequencies of the A^3 and B alleles of ß-casein revealed in our Holstein-Friesian bulls are similar to those quoted in the literature. The higher frequency of the A^3 allele in our high-yielding cows (0.05) may indicate that the correlation observed by Ng-Kwai-Hang *et al.* (1984) is significant. The linkage disequilibrium between ß-casein and κ-casein loci which we have confirmed stresses that these loci cannot be selected individually, rather for effective selection it is the favoured haplotype at the casein loci which will be the selection objective.

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