GENOTYPING THE κ-CASEIN LOCUS IN CATTLE USING PCR

C.J. Skidmore, S.J. Pinder, B.N. Perry* and D. Savva
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 the *Hin*dIII polymorphism in the bovine κ-casein gene, to ascertain the genotype of cattle at this locus. Blood and semen can be used as sources of DNA for the test, although there is some ambiguity in the typing of twins from blood. This test can be used to select for animals carrying the B allele of κ-casein, which is favoured for cheese production.

INTRODUCTION

There is considerable phenotypic variation in milk proteins, which derives from both substitutions and deletions in their amino acid sequences (Eigel *et al.*, 1984). κ-casein, which plays a major role in stabilising the casein micelle, has two major variants (A and B). This variation has a marked effect on the processing quality of milk: the B-variant possesses the better coagulation parameters (Schaar, 1984; Pagnacco & Caroli, 1987).

The changes in amino acid sequence which define the A and B variants are the result of nucleotide sequence changes in the κ-casein gene which also give rise to a *Hin*dIII restriction fragment length polymorphism (RFLP). DNA from the B allele is cleaved by *Hin*dIII whereas that from the A allele is not: this RFLP has been shown to be informative by our group and others (Leveziel *et al.*, 1988; Damiani *et al.*, 1989; Perry *et al.*, 1989).

We have now developed a more rapid and sensitive test based on this RFLP but utilising the polymerase chain reaction (PCR; Saiki *et al.*, 1988) which can genotype cattle at the κ-casein locus from a small sample of blood or semen.

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. Yields were usually in the range of 20-40 μg DNA/ml blood and 40 μg DNA/μl semen.

Amplification of DNA: PCR was used to amplify the DNA between nucleotides 10592 and 11466 of the κ-casein gene (Alexander *et al.*, 1988). Each reaction (100μl) contained 100ng DNA in 10mM Tris-Cl, pH 8.3, 50mM KCl, 1.5mM $MgCl_2$, 0.1% (w/v) gelatin, 200μM of each dNTP, 1μM of each of the two oligonucleotide primers (5'-GTGCTGAG(T/C)AGGTATCCTAG-3' and 5'-GTAGAGTGCAACAACACTGG-3') and 2.5 units

*Present address: AFRC Institute of Food Research (Reading Laboratory), Shinfield, Reading RG2 9AT, UK

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 57°C, and 3 min at 74°C.

Enzyme digestion: Aliquots of each PCR reaction were incubated at 37°C with 10 units *Hin*dIII and the products analysed by electrophoresis on 1.4% (w/v) agarose gels and stained with ethidium bromide.

RESULTS

We have amplified an 874 bp fragment from the third exon of the κ-casein gene (Alexander *et al.*, 1988). In the A allele this contains no site for *Hin*dIII: the B allele is cleaved by *Hin*dIII into two fragments of 521 and 353 bp (Figure 1). Following PCR, enzyme digestion and gel electrophoresis, DNA from AA homozygotes shows a single band at the same position as uncut DNA, BB homozygotes two faster running bands and heterozygote animals give all three bands.

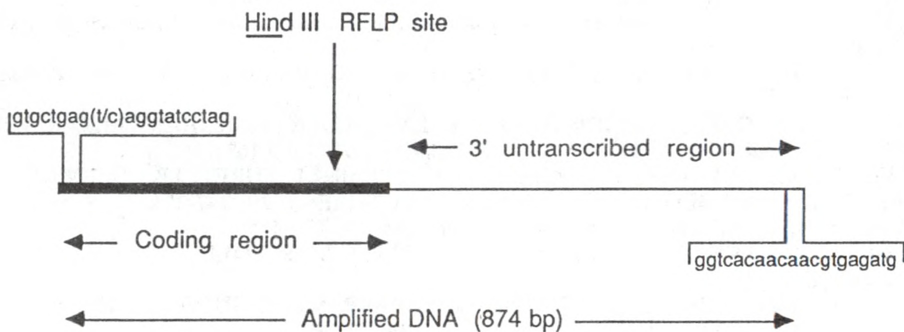

Figure 1 Diagram of the PCR test for κ-casein

Using this method we have determined the κ-casein genotype of 23 bulls and 28 cows. Of these animals, 16 bulls and 3 cows have also been analysed by Southern blotting and hybridisation. In all cases there was complete agreement between the genotypes obtained by the two methods. In one case, the genotype obtained from DNA (AB) disagreed with the phenotype derived by electrophoresis of milk proteins (AA). This cow was a twin whose sibling was an AB heterozygote. The B allele DNA (obtained from a blood sample) must have derived genetically from the twin and been transferred *via* shared placentation. The blood cell chimaerism which occurs in bovine twins can produce false genotypes which have to be confirmed at the phenotypic level.

This PCR method takes less than a day to determine the κ-casein alleles present in a DNA sample and is thus the method of choice for large-scale screening of cattle populations. In the 51 cattle analysed by the PCR, there were 35 AA, 12 AB and 4 BB animals (gene frequencies of 0.80 and 0.20 for the A and B alleles).

DISCUSSION

The genotype of cattle at the κ-casein locus can be ascertained using the PCR RFLP test described here. This typing can be performed using easily available sources of DNA, ie. blood and semen, and is thus effective for animals of any age or sex. Aside from the problem of twin animals genotyped from blood as heterozygotes (also observed by Leveziel *et al.*, 1988) which require a retest using another tissue, the genotype is predicted with complete assurance.

This information will allow producers readily to select for cattle bearing the more favourable B allele of κ-casein. We are currently collaborating with animal breeders in such a study and are also studying the incidence of the two alleles in a much larger population of proven and unproven AI stud dairy bulls. We shall be particularly concerned to show that cattle of the BB genotype do not carry other inferior traits which would preclude their use in breeding.

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