

GENETIC VARIATION IN A DINUCLEOTIDE REPEAT IN THE K-CASEIN GENE IN CATTLE (*BOS TAURUS*).

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SUMMARY

The casein genes have been of special interest regarding association with production characters. This paper describes the genetic variation in a microsatellite in the κ -casein gene in different Danish breeds. A total of 5 alleles was found in 92 unrelated animals from different beef and dairy cattle breeds. The beef breeds showed a higher number of alleles than the dairy cattle indicating a loss of genetic variation in the dairy breeds.

INTRODUCTION

Several investigations have shown an association of the casein genes to production traits. The different genotypes of the genes influence milk yield, fat and protein yield (Ng-Kwai-Hang et al., 1984; Aleandri et al., 1990).

The casein genes are situated within 200 kb of chromosome 6 (Ferretti et al., 1990). The alleles of the casein genes behave like haplotypes because of this tight linkage. These haplotypes are then related to the production characters. Further subdivision of the casein haplotypes might reveal specific types with stronger association to the production characters than revealed by genotyping each casein gene.

A search for repeat sequences in the casein genes was performed to investigate the possibility of further variation in the casein region. This paper reports the occurrence of repeat sequences in the α_{s2} - and κ -casein genes.

METHODS

A total of 92 animals belonging to different breeds were surveyed. The breed distribution can be seen in table 1. All animals were considered unrelated as they had no grandparents in common. DNA was extracted from either blood or semen samples using a salt precipitation procedure (Miller et al., 1988).

A survey of the sequences in the EMBL database using FASTA (Devereux et al., 1984) showed a (TGC)₈ sequence in the α_{s2} -casein gene (EMBL acc.no. M94327) and a compound repeat with the sequence (TA)₁₀(CA)₆N₃₅T₇ in the κ -casein gene (EMBL acc.no. X14908). To investigate the genetic variation in these microsatellites, PCR primers were designed using the software package OLIGO. One of the primers of each primer set was end-labeled in the 5' end with fluorescein. The primer sequences were:

α_{s2} -casein: 5'-GTAGGCTGCAGTAGATGGGAT-3'
5'-GAACACAGAGGAGAGGGATAT-3'
 κ -casein: 5'-TGACATACAATACACAAGCATAAC-3'
5'-CAACATATAAACCCAGGAATC-3'

The PCR reaction contained approx. 40 ng genomic DNA, 100 μ M dNTP, 14 mM Tris-HCl, 70 mM KCl, 2.1 mM MgCl₂, 0.14% Triton X-100, 0.5 μ M of each primer and 0.4 U Taq Polymerase in a total volume of 25 μ l. The reaction was performed with an initial 3 minute denaturation at 95°C followed by 28 cycles of 1 minute at 94°C, 1 minute at the annealing temperature and 10 seconds at 74°C. The annealing temperature was 56°C with the α ₂-casein primers and 48°C with the κ -casein primers.

The PCR products were analyzed on denaturing polyacrylamide gels using an "A.L.F." DNA Sequencer from Pharmacia. Internal size markers were used in each lane to ensure a uniform typing of the alleles. The sizes of the different alleles were determined using the software package "Fragment Manager" from Pharmacia.

RESULTS

The α ₂-casein repeat showed no variation.

The κ -casein repeat showed considerable variation. The frequencies and sizes of the different alleles in the different breeds are given in table 1.

Table 1. Allele frequencies of the κ -casein microsatellite alleles in different Danish cattle breeds. Het is the observed heterozygosity and N is the number of animals surveyed.

Breed	Allele (size in bp)					Het	N
	239	241	245	247	251		
Danish Black-and-White	0.03	0.30	0.67	-	-	0.46	15
Danish Jersey	-	0.69	0.31	-	-	0.43	13
Danish Red	0.17	0.25	0.58	-	-	0.57	12
Charolais	0.11	0.72	0.11	0.06	-	0.45	9
Blonde d'Aquitaine	-	0.79	0.21	-	-	0.34	7
Limousin	-	0.67	0.29	0.04	-	0.47	12
Simmental	0.04	0.46	0.42	-	0.08	0.60	13
Hereford	-	0.33	0.67	-	-	0.44	6
Aberdeen-Angus	0.20	0.40	0.30	-	0.10	0.70	5
Dairy breeds	0.06	0.41	0.53	-	-	0.55	40
Beef breeds	0.05	0.58	0.33	0.02	0.03	0.56	52

DISCUSSION

In dinucleotide repeats the probability for detecting variation in the repeat number is low when the number of repeats is below 15 (Weber & May, 1989). The lack of variation in the trinucleotide repeat in the α ₂-casein gene confirms this observation for trinucleotide repeats.

The alleles 247 and 251 are only present in the beef breeds. This may be the result of selection in the dairy breeds for milk composition and/or milk production or it may be an effect of a small effective population size sometime during the past. A bottleneck will normally result in the loss of rare alleles but will not lower the populations level of heterozygosity unless the bottleneck lasts for a long period or is extremely severe. Selection for milk production traits will also result in a risk of losing alleles if these alleles are linked to unfavourable alleles from other loci.

The 239, 241, and 245 alleles have all been found in κ -casein AA animals in Danish Black-and-White cattle (unpublished results). This indicates that it may be possible to subdivide the different haplotypes of the casein region by using the microsatellite alleles.

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