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Note

Polymorphism of β-casein in the Creole goat of Guadeloupe: evidence for a null allele

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Summary – A polymorphism of β-casein, including several null phenotypes, was observed in a large flock of Creole goats of Guadeloupe. In addition to the common allele, β-Cn^A, this polymorphism includes 2 new alleles: β-Cn^B with a frequency of 0.03 and β-Cn^O, a null allele with a frequency of ≈ 0.2. The null allele was found in 2 different α_{s1}-Cn, β-Cn haplotypes: α_{s1}-Cn^B, β-Cn^O and α_{s1}-Cn^A, β – Cn^O. This suggests the possible existence of 2 different mutations producing a null allele at locus β-Cn.

goat / β-casein / polymorphism / null type

Résumé – Polymorphisme de la caséine β dans un troupeau de chèvres créoles de Guadeloupe : mise en évidence d'un allèle nul. *Un polymorphisme de la caséine β, comprenant entre autres plusieurs phénotypes nuls, a été observé dans un grand troupeau de chèvres créoles de Guadeloupe. Ce polymorphisme s'explique par la présence, en plus de l'allèle commun, β-Cn^A, de 2 « nouveaux» allèles: β-Cn^B (fréquence : 0,03) et β-Cn^O, un allèle nul (fréquence d'environ 0,2). L'allèle nul a été trouvé dans 2 haplotypes* α_{s1}*-Cn, β-Cn différents :* α_{s1}*-Cn^B, β-Cn^O et* α_{s1}*-Cn^A, β-Cn^O, ce qui suggère l'existence possible de 2 mutations de type nul au locus β-Cn.*

chèvre / caséine β / polymorphisme / allèle nul

INTRODUCTION

Among the 4 types of casein, 3 have been found to be polymorphic in the goat, namely α_{s1-}, α_{s2-}, and κ-caseins. Boulanger *et al* (1984) were the first to describe the polymorphism of α_{s2}-casein with 2 alleles, α_{s2}-Cn^A and α_{s2}-Cn^B, the first being predominant in the Alpine and Saanen breeds. Later studies in other breeds

indicated that this polymorphism was widely distributed (Grosclaude and Tucker, 1992). The polymorphism of α_{s1}-casein, also disclosed by Boulanger *et al* (1984), was further investigated by Grosclaude *et al* (1987) and Mahé and Grosclaude (1989), who established the existence of at least 7 alleles, α_{s1}-Cn^{A,B,C,D,E,F} and ^{O}. Alleles α_{s1}-Cn^{D,E} and ^{F} are considered as defective mutants in that they are associated with less α_{s1}-casein in milk than the normal or strong alleles, α_{s1}-Cn^{A,B} and ^{C}. The characterization of the 6 protein variants was carried out by Brignon *et al* (1989, 1990). Furthermore, it is now suggested that the decreased rate of α_{s1}-casein synthesis associated with allele α_{s1}-Cn^{F} is due to altered RNA splicing, as a consequence of an exonic point deletion (Leroux *et al*, 1992). The 7th allele α_{s1}-Cn^{O}, is a null allele. In the Alpine and Saanen breeds, the defective alleles $\alpha_{s1}-Cn^{E}$ and α_{s1}-Cn^{F} are largely predominant, but in other European breeds, the 'strong' alleles have the highest frequencies (Grosclaude and Tucker, 1992). The existence of a polymorphism of κ-casein (alleles κ-Cn^{A} and κ-Cn^{B}), as first suggested by Russo *et al* (1986), was confirmed by Di Luccia *et al* (1990).

Upon electrophoresis, the 4th type of casein, β-casein, reveals the presence of 2 dark and 1 lighter bands. This heterogeneity, as observed in sheep β-casein (Richardson and Mercier, 1979), is probably due to a different degree of phosphorylation and is not truly a genetic polymorphism. Until now, β-casein has been considered to be monomorphic in the goat. However, Mácha (1981) made reference to 4 alleles in the Czech White Shorthaired breed but no description was given for this polymorphism. In the Italian Garganica breed, Dall'Olio *et al* (1989) found a milk sample with no visible electrophoretic band corresponding to β-casein, but the genetic basis of this phenomenon was not investigated.

We describe here a polymorphism of β-casein found in the Creole goat of Guadeloupe. This population, which is used for meat production, is supposed to have originated from importations taking place in the *17*th to *19*th centuries from Eastern Africa and India (Chemineau *et al*, 1984).

MATERIALS AND METHODS

Individual milk samples were collected from Creole goats bred in the INRA experimental flock (130–150 females) of Gardel, near Le Moule, Guadeloupe (French West Indies). Except for isoelectric focusing (IEF), all other analytical techniques were as described by Grosclaude *et al* (1987).

Isoelectric focusing was performed according to an adaptation of the procedure of Seibert *et al* (1985). IEF was carried out in 5% polyacrylamide gels containing 8M urea, and a mixture of ampholytes (Pharmacia) consisting of: 1.2% (v/v), pH 4.2–4.9; 0.9% pH 2.5–5, 0.3% pH 5–6.5. The skim milk samples were diluted with 4 volumes of distilled water and applied close to the anodic side of the gel. Electrofocusing was carried out with a Multiphor II apparatus (Pharmacia-LKB) in 0.5 mm thick gels (240 × 115 mm). After prefocusing at 14°C and constant power (9 W), focusing was continued for 80 min at 20 W. During the run, the voltage rose from 350 V to 2 500 V. Gels were stained for 10 min in a solution containing 0.2% (v/v) Coomassie blue G-250, 50% methanol and 10% acetic acid in water. Destaining was carried out in an aqueous solution of 30% methanol, 8% acetic acid and 10% glycerol until the background was clear.

RESULTS

Among the 127 females present in the flock in 1989, 6 lacked the β-casein fractions in the electrophoretic pattern of their milk as exemplified by sample 7 in figure 1. Because 3 of the 'null' individuals were offspring of the same male, No 15, the progeny of this sire was used to further investigate the inheritance of the 'null' trait. The β-casein contents of the milk from 15 available daughters, estimated by rocket immunoelectrophoresis, are given in figure 2. In addition to the previously-mentioned 3 'null' individuals, 5 more daughters were considered as having inherited the 'null' trait. Assuming that this character originated from the sire (because the dams involved were no longer available to check this inference) the 8:7 ratio, which is not different from the mendelian 1:1 ratio, suggests the existence of a null allele, $\beta\text{-Cn}^{O}$, at the β-casein locus. Based on the proportion of homozygous individuals in the flock, a rough estimate of the frequency of $\beta\text{-Cn}^{O}$ would thus be 0.2 ($\sqrt{6/127}$). Taking this value as the probability of transmission for allele $\beta\text{-Cn}^{O}$ by the dams, the expected proportions of the 3 genotypes among the progeny of male No 15 would be: $\beta\text{-Cn}^{O/O} = 1.5$; $\beta\text{-Cn}^{A/O} = 7.5$; $\beta\text{-Cn}^{A/A} = 6$ which are not statistically different from the observed figures of 3:5:7.

It is known that the 4 bovine casein loci are closely linked (Grosclaude, 1979). In the goat, Grosclaude *et al* (1987) concluded that α_{s1}-Cn and α_{s2}-Cn were also linked, whereas the status of β-Cn and κ-Cn could not be investigated due to the absence of detectable polymorphism in the latter 2 caseins. In the family of male No 15, allele $\beta\text{-Cn}^{O}$ appears to be transmitted together with $\alpha_{s1}\text{-Cn}^{B}$, while $\beta\text{-Cn}^{A}$ is transmitted with $\alpha_{s1}\text{-Cn}^{A}$ (4 informative daughters for each case). The inheritance of $\beta\text{-Cn}^{O}$ could be further studied in the small families (3–5 offspring) of 4 heterozygous males originating from No 15 (fig 2). Again $\beta\text{-Cn}^{O}$ was transmitted in association with $\alpha_{s1}\text{-Cn}^{B}$ (5 informative daughters). As could be expected, these results confirm that, in the goat, locus β-Cn is linked to α_{s1}-Cn and α_{s2}-Cn, as in cattle. However, the existence of a second haplotype including $\beta\text{-Cn}^{O}$, $\alpha_{s1}\text{-Cn}^{A}$, $-\beta\text{-Cn}^{O}$, was ascertained in another family (male No 111).

In the same flock, 8 individuals had additional 'new' β-casein patterns. In 1 case (fig 1, sample 6) the β-casein bands were lighter than normal, and were markedly closer to the cathodic position. In the 7 other cases, the same bands were observed together with the usual β-casein fractions (fig 1, sample 5). This suggested the existence of an additional β-casein allele, $\beta\text{-Cn}^{B}$, a hypothesis supported by the segregation observed in the only available family (male No 7004, transmitting $\beta\text{-Cn}^{B}$ to 3 of its 5 offspring). According to family data, the genotype of sample 6 is $\beta\text{-Cn}^{B/O}$, which explains the lighter appearance of β-casein fractions. The frequency of allele $\beta\text{-Cn}^{B}$ in the flock was 0.03.

In starch gel electrophoresis, the β-casein B bands migrate faster under alkaline pH and slower in acid pH than those of β-casein A. In both conditions, their position is shifted to a distance equivalent to the charge of one phosphate group (not shown). These pecularities suggest that the difference in mobility between $\beta\text{-Cn}^{A}$ and $\beta\text{-Cn}^{B}$ is due to an extra phosphate group in $\beta\text{-Cn}^{B}$.

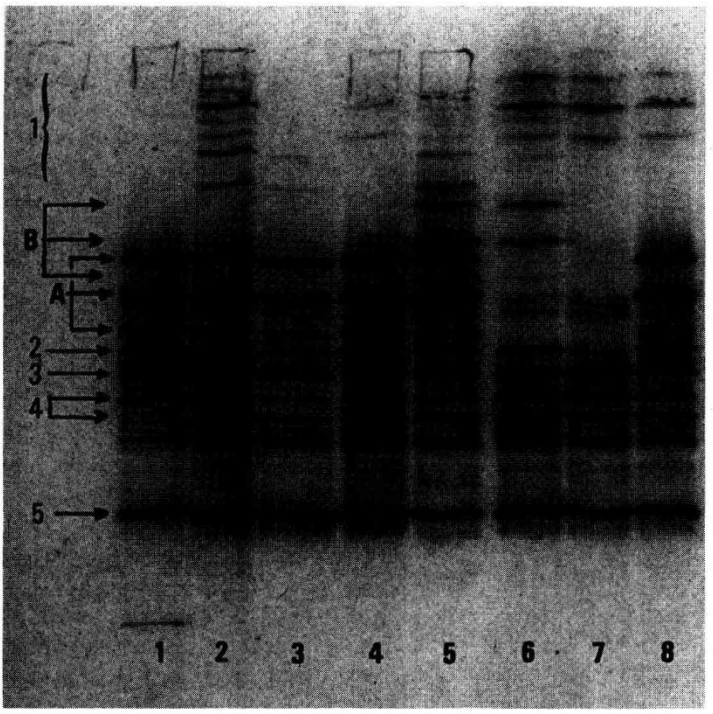

Fig 1. Isoelectric focusing pattern of 8 individual milk samples from Creole goats. The β-casein fractions corresponding to alleles β-Cn^A and β-Cn^B are indicated by A and B. The β-casein genotypes of samples 1–8 are as follows: 1– 4, and 8: β-$Cn^{A/A}$; 5: β-$Cn^{A/B}$; 6: β-$Cn^{B/O}$; 7: β-$Cn^{O/O}$ (null genotype). Numbers located on the left of electrophoretic bands indicate the other milk protein fractions: 1: α_{s1}-casein; 2: κ-casein; 3: α-lactalbumin; 4: α_{s2}-casein; 5: β-lactoglobulin. The α_{s1}-casein genotype of sample 7 is α_{s1}-$Cn^{B/B}$; animal 7 was thus homozygous for the haplotype α_{s1}-Cn^B, β-Cn^O. The poor resolution of the α_{s1}-casein bands for some samples (1,3 and 4) is due to the low content of α_{s1}-casein in those milks.

DISCUSSION

Allele β-Cn^A was the only one found in the already investigated breeds. The polymorphism of β-casein observed in the Creole goat of Guadeloupe is controlled by 2 additional alleles, β-Cn^B and β-Cn^O. Although infrequent, the null allele β-Cn^O may be widespread, since a null individual was observed in the Italian Garganica dairy breed (Dall'Olio *et al*, 1989), and another in the local dairy population of Corsica, France (MF Mahé, 1991, unpublished results). It remains to be established whether all the observed cases were derived from one single or from several mutational event(s).

The existence in the Creole population of 2 different haplotypes including β-Cn^O could either be due to the occurrence of 2 independent 'null' mutations, or to

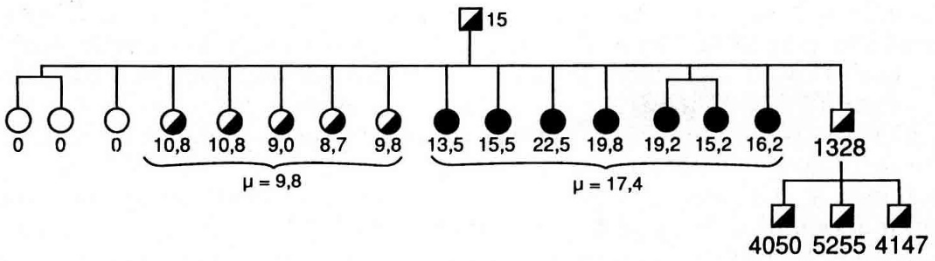

Fig 2. Family of male No 15. Each circle is for a female offspring. The figures below are estimates of the β-casein content (g/l) of their milk. Their genotypes are interpreted as follows: light circles: $\beta\text{-Cn}^{O/O}$; dark circles: $\beta\text{-Cn}^{A/A}$; composite circles: $\beta\text{-Cn}^{A/O}$. The squares are for a son and 3 grandsons, all having the genotype $\beta\text{-Cn}^{A/O}$.

1 single mutation, transferred into a second haplotype by recombination. In cattle, linkage disequilibrium between alleles of the α_{s1}-Cn and β-Cn loci is particularly strong (Grosclaude, 1979). Most probably, the situation in the goat is similar and consequently, one would be inclined to favour the hypothesis of 2 different mutations. This question may be considered in the light of what is presently known about the null allele of goat α_{s1}-casein, $\alpha_{s1}\text{-Cn}^{O}$, found by Grosclaude *et al* (1987). In this case, recent DNA studies have established that there are in fact 2 different α_{s1}-casein null alleles, $\alpha_{s1}\text{-Cn}^{O_1}$, and $\alpha_{s1}\text{-Cn}^{O_2}$, characterized by clearly different mutations (C Leroux and Y Amigues, personal communication).

The null β-casein allele is the sixth example of a defective mutant in the cluster of goat casein loci, in addition to the 5 already identified at the α_{s1}-Cn locus ($\alpha_{s1}\text{-Cn}^{D,E,F,O_1,O_2}$). The reasons for such an accumulation of defective alleles in the goat, which is not observed in the cow, remain unclear.

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