GENETIC POLYMORPHISMS AT THE *K*-CASEIN *LOCUS* IN PANTANEIRO CATTLE

POLIMORFISMOS GENÉTICOS EN EL *LOCUS* DE LA *K*-CASEÍNA EN GANADO BOVINO DE RAZA PANTANEIRA

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Genetic variability. Heterozygosity. Genetic resources.

PALABRAS CLAVE ADICIONALES

Variabilidad genética. Heterocigosidad. Recursos genéticos.

SUMMARY

First results on genetic variability at the κ-casein *locus* are reported for the Pantaneiro breed of cattle, based on DNA analysis by PCR-RFLP of blood samples from 117 animals. Estimated gene frequencies were 0.7820 ± 0.0281 and 0.2180 ± 0.0281 for the A and B alleles, respectively, with an observed heterozygosity of 0.368. Based on information at this *locus*, no evidence was found of disequilibrium in the population. Comparison with allele frequencies in other cattle breeds indicates that frequencies in Pantaneiro are intermediate between those observed in *Bos taurus* and *Bos indicus* breeds.

RESUMEN

En este estudio se presentan los primeros resultados sobre la variabilidad genética en el *locus* de la κ-caseina en ganado de raza Pantaneira. Se analizaron 117 muestras de ADN mediante las técnicas de PCR-RFLP. Las frecuencias obtenidas para los alelos *A* y *B* fueron 0,7820 ± 0,0281 y 0,2180 ± 0,0281, respectivamente, y la heterocigosidad observada fue de 0,368. Con base en la información sobre este *locus*, no se encontró evidencia de que la población no estuviera en equilibrio. Las frecuencias alélicas que fueron estimadas en Pantaneiro son intermedias entre aquellas obtenidas en razas de origen *Bos taurus* por un lado y *Bos indicus* por el otro.

INTRODUCTION

Selection of dairy sires and cows has been based mostly on quantitative traits such as milk, fat or protein yield, which are assumed to be controlled by multiple *loci*. Genetic improvement of quantitative traits is, therefore, relatively slow, as productive traits can only be measured in one sex, are affected by numerous polygenes (each polygene exerting a small effect on the trait) and environmental factors have an important influence on their expression. This undoubtedly lowers the accuracy of genetic evaluation of sires and cows. In addition, productive traits can only be measured in adult animals, thereby increasing the generation interval and lowering the genetic progress per year.

For this reason, qualitative characters, such as polymorphisms in blood groups, enzymes, blood serum proteins or milk protein types, are among those being investigated for the possibilities which they provide of improving the accuracy of estimating genetic merit of sires and cows and practicing selection at an earlier age (Lin *et al.*, 1992). More recently, genome scanning based on microsatellite loci has also been used to detect the possible location of genes with a major effect on productive traits (Boichard, 1998; Gomez-Raya *et al.*, 1998).

Milk protein polymorphisms have received considerable research interest because of their potential use as an aid to genetic selection and to genetic characterization of bovine breeds (Del Lama and Zago, 1996; Golijow *et al*,. 1996 and 1999; Tambasco, 1998; Kemenes *et al.*, 1999).

Specific proteins in bovine milk include four caseins (αs1, αs2, β and κ-casein), and two whey proteins (α-lactalbumin: α-La, and β-lactoglobulin: β-Lg), each protein showing at least two genetic variants (Eigel *et al*., 1984). The κ-casein variants *A* e *B* differ by two amino acid substitutions, i.e., Thr136/Ile and Asp148/Ala (Lin *et al.*, 1992)

Several studies have reported that some of these bovine protein variants, particularly certain κ-Cn and β-Lg variants, are associated with lactation performance and have a major influence on milk composition and its processing properties, including cheese yield (Marziali and Ng-Kwai-Hang, 1986; Grosclaude, 1988, Aleandri *et al.*, 1990).

Relationships between genotypes for different milk proteins and yield traits have been reported by several authors (Lin *et al.*, 1992). For example, associations between milk protein genotypes and milk production from 6803 first lactation records were estimated by Bovenhuis *et al.* (1992). In this study, κ-casein genotype had a significant effect on milk production ($p<0.001$), with cows of the BB genotype producing 173 kg less milk than AA cows. Furthermore, κ-casein genotypes had a highly significant effect on protein content ($p<0.001$), such that κ-casein BB cows produced milk with a 0.08 percent higher protein content than that of AA cows. The same authors found that, for fat content, the effect of κ-casein genotype was not significant, while for fat yield the *B* allele was associated with a significantly lower production of fat when compared with the *A* allele.

Genotyping of milk proteins, such as κ-casein, can be performed by electrophoresis, directly from milk samples, as the expression of caseins occurs only during the lactation phase in mammary gland cells. Therefore, the use of electrophoresis for genotyping of milk proteins is strongly limited because it can only be used in cows in the lactation stage. With newly developed techniques based on DNA analysis, which include polymerase chain reaction and restriction fragment length polymorphisms (PCR-RFLP) methods (Medrano and Aguilar-Cordova, 1990; Denicourt *et al.,* 1990), it is now possible to determine the k-casein genotype of all individuals in a given population under selection, regardless of sex, age or physiological stage. As a result, it is now possible to include information on milk protein genotypes into selection programs, which should result in more accurate predictions of breeding values of animals to be selected, and thus improve response to selection.

Genetic variability in the κ-casein *locus* has been reported for several breeds, with allelic frequencies incorporated into studies on genetic diversity among breeds (Golijow *et al.,* 1996; Del lama e Zago, 1996; Kemenes *et al.,* 1999).

The Pantaneiro breed of cattle represents an excellent source of biological information for studies on genetic characterization, as it results from a long process of natural selection. This breed has been kept basically with no selection for productive traits, and it should therefore maintain the genetic variability which has allowed it to adapt and survive in the adverse conditions of the Pantanal region.

The objective of this work was to study gene frequencies at the κ-casein *locus* in Pantaneiro cattle, and compare them with those reported for different commercial cattle breeds.

MATERIALS E METHODS

Peripheral blood was sampled from 117 cattle of the Conservation Nucleus for Pantaneiro cattle of EMBRAPA Pantanal. DNA was obtained by digestion with proteinase K and salt extraction (Olerup and Zetterquist, 1992) and DNA samples were typed using the PCR-RFLP method.

To analyze the κ-casein (κ-CN) *locus*, a 350-bp fragment covering the sequence containing the mutation site was amplified according to the procedure proposed by Medrano and Aguilar-Cordova (1990). The amplicon was digested with *HinfI* restriction endonuclease at 37^0C for three hours, to distinguish between *A* and *B* alleles. For each reaction, 10µl of PCR product and 3.75 units of *HinfI* were used in 50 mM Tris-HCl buffer, pH 8,0, containing 10 mM $MgCl_2$ and 50 mM NaCl. The restriction fragments were separeted in vertical polyacrylamide gel 10 percent and silver stained at 3 percent.

Allele frequencies were determined by gene counting. A Chi-square test was carried out to evaluate if the population was in Hardy-Weinberg equilibrium.

RESULTS AND DISCUSSION

Identification of *A* and *B* alleles of κ-casein was performed by amplification of a DNA fragment of 350-bp, located between exon IV and intron IV, by the PCR-RFLP method. The DNA fragment amplified from allele *B* shows only one restriction site, resulting in two fragments of 266 and 84 bp. Allele *A* was characterized by the presence of three fragments, corresponding to 134, 132 and 84-bp. The patterns of the three possible κ-casein genotypes are shown in **figure 1**.

Genotypic frequencies were 0.598, 0.368 and 0.034 for AA, AB and BB, respectively. Frequencies of alleles *A* and *B*, estimated from genotypic frequencies, were 0.7820 ± 0.0281 and 0.2180 ± 0.0281, respectively **(table I)**. Genetic variation at the κ-casein locus has not been previously reported for Pantaneiro cattle, but the observed heterozygosity indicates a high genetic variabilility in this population, in agreement with the results reported for the same breed by Lara (1998) based on eight protein *loci*.

Deviations between observed genotypic frequencies and those expected under Hardy-Weinberg equilibrium were not significant ($p>0.05$), suggesting that the Pantaneiro cattle population sampled is in equilibrium for the K-Cn *locus*, i.e., there is no selective advantage for any of the genotypes.

Estimated gene frequencies for the K-Cn *locus* reported for different breeds are shown in **table I**. In studies of genetic characterization of cattle breeds, it has been found that the B allele of κ-Cn occurs at higher frequencies in breeds originating from *Bos taurus* than in those of *Bos indicus*

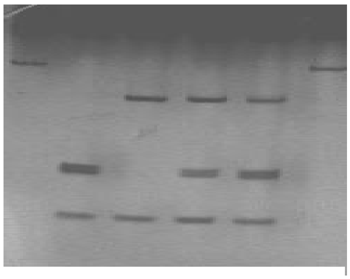

Figure 1. *Patterns of restriction fragments of κ-casein after digestion with* Hinf I. *Lanes **1** and **6:** undigested PCR product; lanes **2**: genotype **AA; 3**: genotype **BB; 4** and **5**: genotype **AB**.* (Patrones de fragmentos de restricción de la κ-caseína después de la digestión con Hinf I. Líneas 1 y 6: producto no digerido por PCR; Líneas 2: genotipo AA; 3: genotipo BB; 4 y 5: genotipo AB).

Table I. *Allele frequencies at the κ-casein* locus *in seven bovine breeds.* (Frecuencia alélica del *locus* de la κ-caseína en siete razas bovinas).

Breeds	κ-Cn alleles		Reference
	A	B	
Pantaneiro	0.7820 ± 0.0281	0.2180 ± 0.0281	
Crioulo da Argentina	0.647	0.353	Golijow *et al.,* 1999
Argentine Holstein	0.656	0.344	Golijow *et al.,* 1999
Jersey	0.112	0.888	Tambasco, 1998
Gyr	0.930	0.070	Kemenes *et al.*, 1999
Rubia Gallega	0.515	0.485	Viana *et al.*, 2000
Nelore	0.910	0.090	Kemenes *et al.*, 1999

origin (Backer and Manwell, 1980; Golijow *et al.*, 1996; Del lama e Zago, 1996; Tambasco, 1998; Kemenes *et al.*, 1999). The fact that the k-Cn *B* allele was detected at a high average frequency in Podolic, Italian and Iberian breeds, a cattle group designated by Backer and Manwell (1980) as primigenius because they present some characteristics which existed in wild cattle (*Bos primigenius*), corroborates the proposition of Del Lama and Zago (1996) suggesting that the κ-Cn *B* allele may be present in the common ancestors of the *Bos* and *Bison* genera.

When results for Pantaneiro are compared with those for other breeds in **table I**, it is apparent that allele frequencies in Pantaneiro are intermediate between those observed for breeds of *Bos taurus* and *Bos indicus* origin. Considering the example of the *B* allele, its frequency was about 0.22 in Pantaneiro, ranged between 0.34 and 0.49 in the majority of *Bos taurus* breeds (Jersey excluded) and between 0.07 and 0.09 in *Bos indicus* breeds.

Tambasco (1998) reported a gene frequency for the *B* allele of κ-Cn of 0.88 in Jersey cattle, which is in agreement with the cheese-making properties attributed to milk produced by this breed, given its high protein content and the curd firmness obtained. According to Marzali and Ng-Kwai-Hang (1986), cheese production can be increased by 10 percent if milk is from cows of the BB genotype for K-Cn, when compared with milk from AA animals.

In the Holstein and Jersey breeds it has been shown that the *B* allele is associated with a higher protein content in milk (Aleandri *et al*., 1990), and it has been suggested that approriate weights could be given to genotypic information and polygenic breeding value in order to improve selection response (Van Arendonk and Bovenhuis, 1996).

In beef cattle, maternal ability is a major factor affecting production efficciency, especially under extensive systems. As milk production is an important component of maternal ability, it would be important to evaluate in beef breeds, particularly those kept in tropical conditions, if a relationship exists between a cow's genotype at the κ-Cn *locus* and her maternal performance, as well as possible associations with other productive and reproductive traits. If that is the case, selection response in beef production systems could be enhanced by including genotypic information in selection decisions.

CONCLUSIONS

Pantaneiro cattle shows a high degree of genetic variability for the κ-Cn *locus*, with a frequency of the *B* allele of 0.218. As this allele has been shown to be favorably related to milk composition in dairy cattle breeds, it can be anticipated that an association may also exist with maternal performance in beef breeds. Therefore, studies aimed at establishing this possible relationship are of crucial importance, as selection could be enhanced by the inclusion of genetic markers in selection decisions.

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