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K-casein Genotypic and Allelic Frequencies in the Tropical Milking Criollo Cattle

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ABSTRACT: The objective of this study was to estimate the genotypic and allelic frequencies of the κ-casein A and B variants and their relationship with milk production and composition of the Tropical Milking Criollo cattle. Blood samples were collected from 180 females and the DNA was obtained. A segment of the κ-casein gen was amplified by PCR–and genotyped by restriction endonuclease digestion. Genotypic and allelic frequencies were .09, .60 and .31 for AA, AB and BB ($p \leq 0.05$) and .39 and .61 for A and B variants ($p \leq 0.05$). For genotypes AA, AB and BB milk production per day, fat and protein percentages were 3.7±.6, 3.9±.4, 4.1±.5; 3.8±.5, 3.6±.5, 3.7±.5; 3.7±.1, 3.7±.4, 3.7±.1, with no statistical differences ($p > 0.05$). Studies on simultaneous effects of the κ-casein and β-lactoglobulin and/or the complete casein complex on milk production and composition seem guaranteed.

Keywords: Tropical Criollo Cattle; K-casein; Milk production

Introduction

The Tropical Milking Criollo cattle (TMC) comprises ancient breeds and strains of the Americas, which descend from cattle brought from the Iberian Peninsula, and are adapted to tropical harsh environmental conditions of high solar radiation, temperature and humidity with a long draught season (de Alba (2011)). Such conditions are related, among other things, to poor forages availability and high incidence of internal and external parasites. Different topics of Mexico´s TMC have been studied, such as resistance to tick infestations, productive and reproductive performance, and genetic parameters and trends on milk production (Rosendo-Ponce and Becerril-Pérez (2002), Santellano et al. (2008), González-Cerón et al. (2009)); also, studies on genotypic and allelic frequencies of the β-lactoglobulin molecular markers related to milk production and composition were published (Meza-Nieto et al. (2010); (2012)). The TMC breed was declared an endangered popu|lation (FAO (2000)) and in Mexico, there are less than 1000 pure animals. This naturalized breed represents a reservoir of genes, product of isolation and 500 hundred years of an adaptation process to hot climates of tropical environmental conditions. In tropical Mexico, there are small milk manufacturers that have only recently started to pay attention to cheese manufacturing and the properties of milk for cheese making.

The genetic polymorphism of the milk proteins influence production and composition (Caroli et al. (2004)). In a single locus study, the B rather than the A variant of the β-lactoglobulin was more frequent in the TMC population, 0.65 vs 0.35, and found to be related to more protein 4.6 ± 0.2^a, 3.6 ± 0.2^b, 3.9 ± 0.2^b; fat 4.4 ± 0.2^a, 3.4 ± 0.2^b, 3.2 ± 0.2^b, and total solids 13.9 ± 0.3^a, 12.3 ± 0.3^c, 12.8 ± 0.3^b ($p \leq 0.05$) in the milk of genotypes BB, AB and AA of TMC cows (Meza-Nieto et al. (2010); (2012)). The κ-casein has 12 variants, being the A and B the most important to milk quality and products (Molina et al. (2003)). de Alba (1997) showed evidence of κ-casein variants on milk composition and cheese yield of TMC cows. K-casein genotypic and allelic frequencies of the TMC cattle from Mexico are unknown, as well as their effect on milk composition. Among protein polymorphism, the κ-casein has been more related to rheological quality of milk and cheese manufacturing. However, interactions with other milk protein systems have to be taken into account, in particular β-lactoglobulin and β-casein (Caroli et al. (2009)). In addition, casein haplotypes effects on milk production and composition have also been studied, considering the complete or most part of the casein complex (Nilsen et al. (2009)). In recent studies on milk genetic markers more complex genomic laboratory analyses are involved (Acosta et al. 2011); though, in this study only single locus analyses were able to perform. The objective of this study was to estimate the genotypic (AA, AB, BB) and allelic frequencies of the κ-casein genetic A and B variants, and their relationship with milk production and composition of Tropical Milking Criollo cows. This work is part of a widest research aimed to study the complex of casein in the TMC breed.

Materials and Methods

Source of data. Data were collected from two TMC herds located in the tropical lowlands of the Gulf Coast of Mexico, 20 km south of the port of Veracruz, at 18º 53´ - 19° 11' N and 96º 15´ - 96° 20' W, and maximum altitude of 32 m above sea level. Climatic conditions are hot sub humid with summer rains and seven months drought season; mean annual temperature ranges between 25.0 and 26.4 °C, and rainfall ranges from 1060 to 1400 mm per year (García (1988)).

Animals grazed on introduced and native pastures of pará (*Brachiaria mutica*), German grass *(Echinocloa polystachia*), pangola (*Panicum maximum*) and amargo (*Paspalum spp*). Cows were manually milked once a day in the morning with the calf at foot.

Blood samples were collected from the coccygeal vein of 180 TMC females (96 cows) and milk lactation samples from 40 cows. In each fresh milk sample (100 mL), bronopol 2-bromo-2-nitrol, and 3 propanediol were added as preservative. The samples were transported at a temperature of 4 °C and stored in the laboratory at –20 °C until their analysis. Milk samples of 50 ml were used for fat and protein chemical analysis by infrared spectroscopy.

DNA purification. The DNA was obtained from the peripheral blood samples by the salting out protocol (Miller (1989)). Integrity of DNA was assessed by electrophoresis in 1% agarose gels stained with ethidium bromide. DNA quantification was performed by spectroscopy at 260/280 nm.

Gene amplification. A fragment of the gene κ-casein gene was amplified by the polymerase chain reaction (PCR) under standard procedures in 50 ul reactions. The primers used in the amplification were: forward 5' ATAGCCAAATATATCCCAATTCAGT 3' and reverse 5' TTTATTAATAGTCCATGAATCTTG 3'. The amplification conditions used an initial step at 94 °C, for 5 min, followed by 30 cycles of 94 °C for 30 s, 54 °C for 30 s and 72 °C for s, and a final extension step of 72 °C for 5 min. The amplification was verified by electrophoresis in 1% agarose gels.

Genotyping. Half of the PCR amplified products were digested independently by either the restriction enzymes Hinf I or Hind III. Incubation was at 37 °C for 24 h. Following digestion, DNA fragments were resolved by electrophoresis on 3 % agarose gels stained with ethidium bromide and visualized on a UV trans illuminator. Each genotype renders a specific banding pattern.

Statistical analyses. The comparison of the phenotypic and allelic frequencies of the genetic variants of the κ-casein was made through a Chi-squared analysis. The effect of the genotype of the κ-casein on milk yield at sampling day (kg), fat (%) and protein (%) was estimated through a mixed linear model (SAS (2010)). The cow´s paternal sire was considered as a random effect and the κ-casein genotype as a fixed effect; as well as herd-year-season and parturition number. The days in milk, in its linear and quadratic form, were used as covariates in the model.

Results and Discussion

Genotypic and allelic frequencies. The frequencies of κ-casein genotypes AA, AB and BB were 0.09, 0.60 and 0.31 ($p \leq 0.05$). Heterozygous individuals occurred twice as much frequent than the BB homozygous. Very similar frequencies of 0.10, 0.62 and 0.28 (Rojas et al. (2011)) and 0.11, 0.56 and 0.33 (Rojas et al. (2009)) were observed in other dairy Criollo breed, the Limonero of Venezuela. In the Carora breed (a composite of Criollo and Brown Swiss) genotypic frequencies were 0.11, 0.60, and 0.29 (Pacheco-Contreras et al. (2011)). In single locus analyses, more protein percentage content in milk was associated to genotype κ-casein BB (Bovenhuis et al. (1992)). In contrast, estimated frequencies in another Criollo breed, the Pantaneiro of Brazil, were 0.60, 0.37 and 0.03; this breed has not been selected for productive traits, and maintains the genetic variability for adaptation and survival in the adverse conditions of the Pantanal region (Lara et al. (2012)). Although, frequencies for the Dutch and Chinese Holstein Frisians were 0.64, 0.33 and 0.03 (Bovenhuis et al. (1992)), and 0.55, 0.29 and 0.16 (Rend et al. (2011)). In the TMC the frequencies for the A and B κ-casein alleles were .39 and .61 ($p \leq 0.05$). Same frequencies were estimated by Rojas et al. (2009). In studies on the Gyr Cebu breed, as low as 0.06 and 0.07 frequencies of the B allele were found (da Silva et al. (1997); Quiroz et al. (2011); Kemenes et al. (1999)).

Milk production and composition. Milk production, fat and protein percentages for κ-casein genotypes are presented in Table 1. No differences were found among κ-casein genotypes for any of these traits ($p > 0.05$) in the TMC. Recently, Meza-Nieto et al. (2012) found higher content of fat and protein in the milk of TMC cows with the BB genotype of the β-lactoglobulin. Higher protein content in the milk of cows with the BB genotype of the κ-casein has been reported in Holsteins (Van Eenennaam and Medrano (1991); Bovenhuis et al. (1992)). In addition, in a single locus study the kappa-casein BB genotype of the Sahiwal cattle of India, showed a greater influence on monthly milk yield, 305-days milk yield, monthly SNF yield and monthly protein yield (Rachagani and Gupta (2008)). Also, joint effects of κ-casein and β-lactoglobulin genotypes have resulted in different milk production and composition (Rojas et al. (2011); Van Eenennaam and Medrano (1991)).

Table 1. K-casein genotypes and milk production and composition in the Tropical Milking Criollo cattle.

Genotype	Milk (kg d^{-1})	Fat (%)	Protein (%)
AA	3.7±.6	3.8±.5	3.7±.1
AB	3.9±.4	3.6±.5	3.7±.4
BB	4.1±.5	3.7±.5	3.7±.1

Conclusions

Results suggest that TMC genotypic and allelic frequencies of κ-casein variants are different from other temperate and tropical dairy breeds; with higher frequency of genotypes AB and BB, and the allele B. Single effects of κ-casein genotypes were not significant in fat and protein content of milk. Studies on joint effect of κ-casein and β-lactoglobulin genotypes and/or the complete casein complex on milk composition of the TMC seem guaranteed.

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