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Allelic frequency of the Kappa-Casein gene in Colombian and creole cattle breeds¤

Frecuencia alélica del gen Kappa-Caseína en bovinos criollos y colombianos

Freqüência do alelo de gene de Kappa-Caseína em bovinos crioulos e colombianos

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Summary

Colombia has one of the most genetically diverse creole cattle populations, with eight creole breeds and two improved creole (Colombian) breeds. A high demand for meat and milk has led to the inevitable selection of highly productive cattle and the introduction of foreign breeds. Unfortunately, these breeds are often ill-suited for tropical conditions. These factors threaten the size of the creole livestock population, which is considered part of Colombia's national heritage. Objective: to estimate the allelic frequencies of the Kappa-Casein gene (CNS3) in Colombian creole cattle breeds (GCC). Methods: a total of 354 blood samples were taken from 30 animals of each of the following breeds: Blanco Orejinegro (BON), Caqueteño (CQT), Casanareño (CAS), Horned Costeño (Costeño con Cuernos, CCC), Chino Santandereano (ChS), Hartón del Valle (HV), Romosinuano (ROM), and Sanmartinero (SM), each representing the 8 established "criollo" (creole) breeds; the Lucerna (LUC) and Velasquez (VEL) representing the two Colombian improved breeds; and Brahman and Holstein as control breeds. DNA was extracted by a salting-out procedure and a 453 bp fragment on chromosome 6 was amplified by PCR. CSN3 alleles were identified using single strand conformation polymorphism (SSCP) and their sequence compared with those of the Genebank for Bos taurus and Bos indicus. Results: higher frequencies for allele variants of CSN3 A (0.39) and B (0.41) were found relative to the frequencies of I (0.038), G (0.095), A_1 (0.025), E (0.006), and N (0.006). The allele of interest (CSN3 B) had a high frequency in the CCC (0.81), ROMO (0.66), CQT (0.55), ChS (0.48), and VEL (0.43) breeds. Conclusions: these findings suggest that Colombian creole breeds

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harbor a high genetic diversity which enriches its gene pool and warrants future conservation efforts to protect its integrity.

Key words: *genetic variants, milk proteins, molecular markers, PCR-SSCP.*

Resumen

Colombia es uno de los países más diversos en recursos genéticos criollos. Posee ochos razas de ganado criollo (GCC) y dos razas de criollo mejorado o razas colombianas. La creciente demanda de alimentos ha generado una forzosa selección de individuos altamente productivos e introducción de razas foráneas (Holstein y Brahman) poco adaptadas a condiciones tropicales, lo que ha puesto en riesgo el tamaño efectivo del ganado criollo, considerado patrimonio nacional. Objetivo: estimar la frecuencia alélica del gen (CNS3) de la Kappa-Caseína en el (GCC). Métodos: se usaron 354 muestras de sangre de ocho razas bovinas criollas (30 individuos por raza): Blanco Orejinegro (BON), Caqueteño (CQT), Casanareño (CAS), Costeño con Cuernos (CCC), Chino Santandereano (ChS), Hartón del Valle (HV), Romosinuano (ROMO) y Sanmartinero (SM), dos colombianas Lucerna (LUC) y Velásquez (VEL) y dos controles Brahman y Holstein. Con el fin de estimar la frecuencia de los alelos k-caseína (k-CN) se amplificó un fragmento de 453 pb para k-CN (cromosoma 6). Los alelos se identificaron mediante la técnica PCR-SSCP. Resultados: se encontró mayor frecuencia para las variantes de k-CN A (0.39) y B (0.41), en comparación a I (0.038), G (0.095), A1 (0.025), E (0.006) y N (0.006). El alelo de interés k-CN B presentó alta frecuencia en las razas CCC (0.81), ROMO (0.66), CQT (0.55), ChS (0.48), y VEL (0.43). Conclusiones: la alta frecuencia del alelo de interés del gen k-CN ratifica al GCC como alternativa viable en esquemas sostenibles de producción de leche de mejor calidad y corrobora la necesidad de evaluación y caracterización de recursos zoogenético, como primer paso para su conservación.

Palabras clave: *marcadores moleculares, PCR-SSCP, proteínas leche, variantes genéticas.*

Resumo

A Colômbia é um dos países mais diversificado em recursos genéticos crioulos, tem oito bovinos da raça nativa e duas raças melhoradas ou raças crioulo colombiano (GCC). A alta demanda por alimentos tem levado a uma seleção forçada das pessoas altamente produtivas e/ou introdução de raças estrangeiras mal adaptados às condições tropicais, que têm prejudicado o tamanho efetivo de animais considerados património crioulo. Objetivo: estimar a frequência do alelo do gene Kappa-Caseína (CNS3) na (GCC). Métodos: foram utilizados 354 amostras de sangue de oito raças nativas (30 indivíduos por raça): Blanco Orejinegro (BON), Caqueteño (CQT), Casanareño (CAS), Costeño con Cuernos (CCC), Chino Santandereano (ChS), Hartón del Valle (HV), Romosinuano (ROM) e Sanmartinero (SM), dois Colombianas Lucerna (LUC) e Velásquez (VEL) e dois controles (Brahman and Holstein). Para estimar a freqüência de alelos de κ-caseína (CSN3), amplificaram um fragmento de 453 pb para CSN3. Os alelos foram identificados por PCR-SSCP. Resultados: encontramos uma maior freqüência de variantes do CSN3 A (0.39) e B (0.41), comparado com I (0.038), G (0.095), A_1 (0.025), E (0.006) e N (0.006). O alelo de interesse CSN3 B apresentou alta freqüência em raças CCC (0.81), ROMO (0.66), CQT (0.55), CHS (0.48) e VEL (0.43). Conclusões: estes resultados sugerem que o CCG é um recurso genético, que abriga uma grande diversidade genética e apoia a necessidade de avaliação e caracterização dos recursos genéticos animais como um primeiro passo para a conservação.

Palavras chave: *marcadores moleculares, PCR-SSCP, proteínas do leite, variantes genéticas.*

Introduction

Caseins represent 80% of bovine milk proteins (Fox and Brodkorb, 2008). The *CSN3* gene in cattle has been extensively studied and plays a role in stabilizing caseins, interacting with β-LG, and determining the physical stability of some milk products during heat treatment (Braunschweig *et al.,* 2000; Fox and Brodkorb, 2008). Multiple variants of the *CSN3* gene have been found, although the A and B alleles are the most common. Ten other allele variants have been described and include the following: A_1, C, E, F^1, F^2 G^1, G^2, H, I, and J (Caroli *et al*., 2009). Of these, the B allele

is associated with milk that has a shorter curdling time, a higher protein percentage, a higher stability during freezing, a greater cheese yield, and a more consistent curd formation (Lunden *et al.,* 1997; Wedholm *et al*., 2006; Heck *et al.,* 2009).

Eight Creole cattle breeds have been recognized in Colombia. These include the Romosinuano (ROMO) and the Horned Costeño (CCC) breeds, which are found in the floodplains and the dry plains in northern Colombia (Atlantic Coast); the Blanco Orejinegro (BON) and the Chino Santandereano (Chino) breeds, which are found in the mild temperate region of the Colombian Andes; the Hartón del Valle (Hartón) breed, which is found in the Cauca River Valley; the Casanareño (CAS) and the Sanmartinero (SM) breeds, which are found in the floodplains and high plains of the Colombian Orinoquía region, respectively; and the Caqueteño (CQT) breed, which is found in the Amazon region of Colombia (Martínez, 2010). In the 1930s and the 1950s, the Lucerna and the Velásquez hybrid bovine breeds were developled. The Lucerna breed originates from the Cauca Valley and is the product of hybridizing the Hartón del Valle, the Holstein, and the Shorthorn breeds. The Velásquez breed originates from the Magdalena Valley and is the product of hybridizing the Romosinuano, the Red Poll, and the Brahman breeds (Pinzón, 1991). Although considered a national resource, these breeds are threatened. Currently their population of 18,231 represents only 0.08% of the country's total bovine population (Martínez, 2010).

Assessments of *CSN3* in all but the HV breed have not been previously reported (Naranjo *et al*., 2007; Díaz *et al.,* 2006). The objective of this study was to estimate the allelic frequencies of the Kappa-Casein gene in eight Creole and two Colombian cattle breeds.

Materials and methods

Type of study

A uniform sample size of 30 animals from each of the BON, CQT, CAS, CCC, ChS, HV, ROMO, SM, LUC, and VEL breeds were sampled in different regions of Colombia. These sampling locations are shown in table 1 and figure 1. Special care was taken to select non-blood related animals. For the control, Brahman and Holstein DNA samples from the National University of Colombia's DNA bank were used.

The BON breed is known for its meat and milk production abilities under unfavorable tropical conditions. Its natural habitat is found in the central and western mountain foothills between 800 and 1,800 m above sea level, with temperatures between 18 and 24 ºC (López *et al*., 2001). In contrast, the CQT breed lives in the Caquetá region with an average annual temperature of 26 °C, and 88% relative humidity (Martínez, 1999). The CAS breed inhabits the floodplains of the Arauca and Casanare provinces. This breed is primarily raised for meat, using traditional extensive production methods (Asocriollo, 2007). In contrast, the CCC breed inhabits the coastal plains with temperatures of approximately 27.5 °C and 1.233 mm average annual precipitation (Wilkins *et al.,* 1993). The CCC breed is considered to be one of the best breeds for milk production in the low-tropics (CORPOICA, 2006). The ChS breed inhabits the Lebrija Valley (Santander province) (MADR-Asocriollo, 2003), and the HV breed inhabits the Cauca River Valley between the river's source in the Colombian Massif and La Virginia region (Risaralda province) and between the hydrological divides of the Central and Western Mountain Ranges (Casas and Valderrama, 1998). Conversely, the ROMO breed is adapted to the Sinú Valley conditions, which has a dry tropical forest (DTF) climate and a mean temperature of 27.5 ºC with 83% relative humidity (Martínez, 1998). The SF breed inhabits the wet tropical and very wet tropical forest sub-regions of the Orinoco, which has aluminum rich soils, a mean temperature of 26 ºC, 87% relative humidity during the rainy season, and 55% relative humidity during the dry season (Martínez and González, 2000). The LUC breed originates from the Lucerna Ranch in Bugalagrande (Cauca Valley province) (Asocriollo, 2007), and the VEL breed originates from the Magdalena River Valley in the Caldas province (Corpoica, 2006).

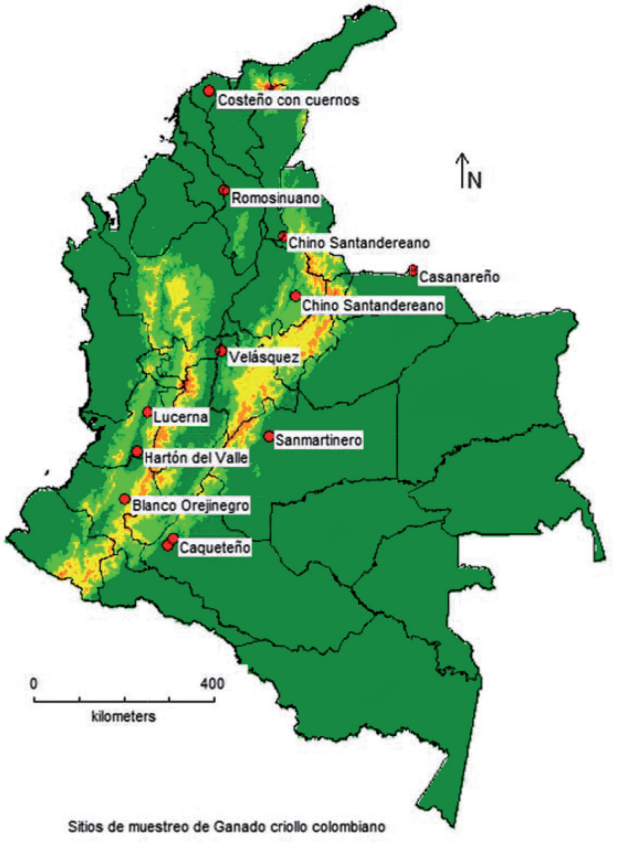

Figure 1. Map of Colombia denoting the sampling sites for Colombian and Creole cattle. This map was produced using the DIVA-GIS software version 7.1.1. (Hijmans *et al.,* 2005).

Table 1. Sample sizes and locations for eight Creole, two Colombian, and two control cattle breeds are listed below.

Breed	N	Location Municipality (Province)
Blanco Orejinegro	30	Popayán (Cauca)
Caqueteño	30	Florencia and Morelia (Caquetá)
Casanareño	30	Arauca (Arauca)
Horned Costeño	30	Campeche (Atlántico)
Chino Santandereano	30	San Gil and San Alberto (Santander)
Hartón del Valle	30	Tuluá, Jamundí, Palmira (Cauca Valley)
Lucerna	30	Cauca Valley
Romosinuano	30	Sincerin (Bolívar)
Sanmartinero	30	San Martín (Meta)
Velásquez	30	La Dorada (Caldas)
Brahman	24	Jamundí, (Cauca Valley)
Holstein	30	Yotoco, Candelaria (Cauca Valley)

Methods

DNA extraction and quantification. The DNA from 5 mL of blood drawn from the coccygeal vein was extracted by using the Salting Out extraction protocol (Miller *et al.*, 1988). The DNA quality was evaluated by using 0.8% agarose gels in a 0.5 X TBE buffer (0.045 M tris-borate, 0.001 M EDTA, pH 8.0) and stained with ethidium bromide. The DNA was quantified by comparing with known Lambda phage DNA concentrations. Electrophoresis was performed at 80 volts (V) for 45 min, and gels were photographed under UV light using a Kodak EDAS 290 digital camera.

CSN3 gene amplification. The 453 bp DNA fragment on chromosome 6 was amplified (Barroso *et al.*, 1998). Between 20 and 50 ng of DNA was mixed with a PCR buffer solution containing 20 pmol/µl of primer (sense, 5´ -TGT GCT GAG TAG GTA TCC TAG TTA TGG-3´; antisense, 5´-GCG TTG TCT TCT TTG ATG TCT CCT TAG-3), 25 mM $MgCl_2$, 1.25 µM of dNTP Mix (MBI fermentas-USA), and 2 U of Taq Polymerase (MBI fermentas-USA) with a total volume of 25 µL. The samples were subjected to denaturation for 5 min, followed by 35 cycles for 1 min at 94 °C, 1 min at 65 °C, and 2 min at 72 °C. At the end, a final 5 min extension step at 72 °C in a PTC-100TM thermal cycler was conducted (MJ Research, Inc-USA).

Identification of alleles. Alleles were identified using SSCP (*Single Strand Conformation Polymorphism*) as described by Barroso *et al.* (1998). Two micro-liters of the PCR product were combined with 8 µl of the denaturing buffer (0.05% xylene cyanol, 0.05% bromophenol blue, 5.5 mM EDTA pH 8.0), denatured at 95 °C for 5 min, and then cooled on ice for 2 min. The samples were loaded into 12 x 28 cm (*Camera Biometra*®, Göttingen, Germany) 12% polyacrylamide gels (acrylamide: N, N´-methylene-bis-acrylamide ratio, 100:1) with 5% glycerol and a 0.5 X TBE buffer (0.045 M tris-borate, 0.001 M EDTA, pH 8.0). The gels were run at 180 V for 12 hours (h) at 12 °C. The resulting bands were stained using silver nitrate. The alleles were verified using PCR-RFLP (*Polymerase Chain Reaction-Restriction Fragment Length Polymorphism*) and the *HindIII*, *HinfI*

HaeIII, HhaI, MaeII, and MspI restriction enzymes. In addition, the alleles were sequenced and compared with the 22 available GenBank sequences and the X14908 (Alexander *et al.*, 1988) GenBank sequence for *Bos taurus,* and with the two available sequences for *Bos indicus.*

Statistical analysis. Allelic frequencies were determined by the direct counting method. The Hardy-Weinberg Equilibrium (HWE) within populations was estimated using the F_{IS} coefficient test (Weir and Cockerham, 1984) and the exact test (Guo and Thompson, 1992). The genetic variability for each breed was calculated from the allele number (AN), the expected heterozygosity (H_E), and the observed heterozygosity (H_O). The coefficient of differentiation ($F_{ST;}$ Weir and Cockerham, 1984) and AMOVA method were used as a measure of genetic subdivision and breed differentiation. All data analysis was performed using the Arlequin Version 3.1 software (Excoffier *et al*., 2006).

Results

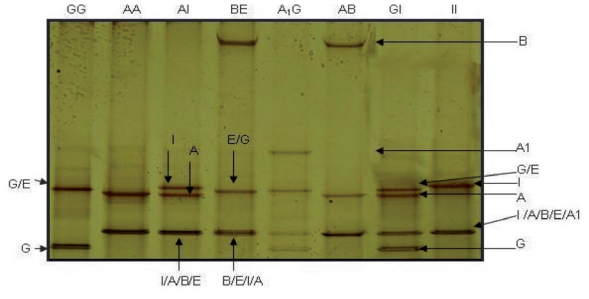

Figure 2. Band patterns for seven allelic variants of *CSN3* in Colombian Creole cattle determined by SSCP are shown. The genotype for each pattern is indicated. Band locations corresponding to each variety are noted on either side. However, note that any given band may belong to different variants.

Seven mobility patterns -corresponding to the A, B, A_1, G, I, and E alleles as well as one allele that could not be identified (N)- were detected using PCR-SSCP. With the exception of the N allele, allele identification was verified through PCR-RFLP and sequencing. Figure 2 shows the detected mobility patterns for the *CSN3* gene belonging to the A, B, A_1, G, I, and E alleles.

Table 2 shows *CSN3* allelic frequencies by breed. The predominant alleles in the GCC population were the B (0.418 ± 0.20) and the A (0.39 ± 0.019) alleles. Other alleles (G, I, A_1, E, and N) had low frequencies and represented only 18% of the total frequency. In contrast, the control breeds had low allelic frequencies for B, with only 0.20 and 0.065 in the Holstein and the Brahman breeds, respectively.

The allele of interest (B) showed greater frequency in the CCC, ROMO, CQT, ChS, and VEL breeds than in the BON, SM, HV, and CAS breeds. The lowest B allele frequency was in the LUC breed. Allele A, which was dominant in the majority of commercial breeds, had a lower frequency in the GCC breed (with the exception of LUC [0.916 ± 0.036] and BON [0.616 ± 0.063]).

The I allele variant was found in six of the eight Colombian breeds and had an average value of 0.038 ± 0.078. In particular, this allele emerged in the SM breed (0.13 ± 0.044). The G allele was detected in eight breeds (0.095 ± 0.01) and had the greatest frequency in the CAS (0.36 ± 0.06), the HV (0.233 ± 0.055), and the VEL (0.116 ± 0.041) breeds. The A_1 allele variant was identified in six breeds (0.025 ± 0.06), but was most present in the CAS breed (0.15 ± 0.046). The E allele variant (0.006 ± 0.003) was found with the same frequency (0.016 ± 0.016) in the CQT, the CAS, the SM, and the VEL breeds. The unidentifiable variant (N) was only present in the ChS and the VEL breeds.

In the HOLS control breed, a greater proportion of the A allele variant was found than the B allele variant. The HOLS control breed also had a low G allele frequency. The A, B, A_1, and G allele variants were detected in the BRAH control breed, of which the G allele was predominant (0.52 ± 0.07).

Table 2. Allelic frequencies and standard deviations for the *CSN3* alleles in eight Creole, two Colombian, and two control breeds are shown below.

	CSN3 Alleles						
Breed	**A**	**B**	**I**	**G**	**A_1**	**E**	**N**
BON	0.616 ± 0.06	0.316 ± 0.06	0.00 ± 0.00	0.050 ± 0.028	0.016 ± 0.016	0.00 ± 0.00	0.00 ± 0.00
CQT	0.25 ± 0.056	0.55 ± 0.065	0.05 ± 0.028	0.066 ± 0.032	0.016 ± 0.016	0.016 ± 0.016	0.00 ± 0.00
CAS	0.10 ± 0.039	0.26 ± 0.057	0.033 ± 0.02	0.366 ± 0.062	0.150 ± 0.046	0.016 ± 0.016	0.00 ± 0.00
ChS	0.283 ± 0.06	0.48 ± 0.065	0.066 ± 0.03	0.066 ± 0.032	0.033 ± 0.023	0.00 ± 0.00	0.066 ± 0.032
CCC	0.15 ± 0.046	0.816 ± 0.05	0.00 ± 0.00	0.033 ± 0.023	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
HV	0.383 ± 0.06	0.30 ± 0.059	0.05 ± 0.028	0.233 ± 0.055	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
LUC	0.91 ± 0.036	0.033 ± 0.02	0.05 ± 0.028	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
ROMO	0.33 ± 0.61	0.66 ± 0.061	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
SM	0.46 ± 0.06	0.316 ± 0.06	0.13 ± 0.044	0.016 ± 0.016	0.016 ± 0.016	0.016 ± 0.016	0.00 ± 0.00
VEL	0.38 ± 0.06	0.43 ± 0.064	0.00 ± 0.00	0.116 ± 0.041	0.016 ± 0.016	0.016 ± 0.016	0.033 ± 0.023
GCC	0.39 ± 0.019	0.418 ± 0.20	0.038 ± 0.078	0.095 ± 0.011	0.025 ± 0.063	0.006 ± 0.003	0.01 ± 0.040
BRAH	0.24 ± 0.063	0.065 ± 0.03	0.00 ± 0.00	0.521 ± 0.074	0.136 ± 0.050	0.00 ± 0.00	0.00 ± 0.00
HOLS	0.76 ± 0.055	0.20 ± 0.052	0.00 ± 0.00	0.033 ± 0.023	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Table 3 shows the number of alleles (NA), observed heterozygosity (H_o), expected heterozygosity (H_e), F_{is}, and the Hardy Weinberg Equilibrium (HWE). The average number of alleles for the GCC breed was 4.60 ± 1.57. A maximum of six alleles were found in the CQT, CAS, ChS, SM, and VEL breeds, and a minimum of two alleles were found in the ROMO breed.

For the GCC breed, the H_e oscillated between 0.16 and 0.74 and had an average H_e of 0.65 ($p<0.05$). The CAS, ChS, HV, VEL, and SM breeds had the highest genetic diversity (H_e), and the LUC and CCC breeds had the lowest. The commercial breeds all had a low H_e. The HWE was not found in the BON, ChS, HV, LUC, and VEL breeds.

The GCC breed showed a low global value for intrapopulational endogamy (F_{IS}) that was highly significant ($F_{IS}=0.098$, $p<0.01$), and was substantially different than zero in the ChS and LUC breeds.

The AMOVA revealed that 80% of genetic variance was due to individual variation, while 20% was due to variation among breeds. The global value for the coefficient of genetic differentiation (F_{ST}) was 0.203 ($p<0.01$). This value indicated that significant genetic differences were distinguishable among the studied cattle breeds.

Table 3. Estimated values for Expected Heterozygosity (H_e), Observed Heterozygosity (H_o), and fixation index (F_{IS}) in eight Creole, two Colombian, and two commercial breeds are shown below.

Breed	NA	H_o	H_e	F_{IS}
BON	4	0.60	0.52*	-0.14
CQT	6	0.65	0.60	-0.08
CAS	6	0.71	0.74	0.03
CCC	3	0.30	0.31	0.05
ChS	6	0.46	0.68*	0.32**
HV	4	0.78	0.69*	-0.13
LUC	3	0.10	0.16*	0.37**
ROMO	2	0.46	0.45	-0.03
SM	6	0.68	0.65	-0.06
VEL	6	0.46	0.66*	0.30
GCC	4.6	0.52	0.65*	0.098
BRAH	4	0.63	0.35	-0.01
HOLS	3	0.30	0.37	0.20

*Statistically significant ($p<0.05$).

**Highly significant ($p<0.01$).

Discussion

According to the Food and Agriculture Organization of the United Nations (FAO, 2007), the inevitable intensification of livestock systems increasingly depends upon the selection of highly productive species. This selection places less-productive but genetically valuable species at risk. As a result, the Colombian Creole SM, ChS, and CQT breeds are in a critical state, the BON, ROMO, HV, CCC, and VEL breeds are in a vulnerable state, and the LUC and CAS breeds are in a threatened state (Martínez, 1999; MADR, 2003). This situation highlights the need to evaluate the diversity of economically important genes in order to enhance their value and assure their conservation.

The alleles detected in this study demonstrated a run pattern similar to that described by Barroso *et al.* (1998), with the exception of the unidentified (N) allele. The majority of studies using PCR-RFLP only identified the A and B alleles. However, Díaz *et al.* (2006) found six alleles in the HV breed and Ceriotti *et al.* (2004) found A, A_1, B, and H alleles in the *Bos indicus* breed and A, B, and E alleles in the *Bos taurus* breed. Likewise, in Nariño province (Colombia), Solarte *et al.* (2009) only detected A and B alleles with PCR-SSCP (1,087 Holstein, Jersey, Normande, Brown Swiss, Simmental, Red Pol, Swedish Red, and Montbeliard cattle and their crosses).

According to Prinzenberg *et al*. (1999), the PCR-SSCP method may introduce genotyping errors because the C allele can be confused with the B allele. Additionally, the E, F, G, H, and A_1 alleles can be confused with the A allele. The latter situation would result in an underestimation of the A allele, an inappropriate rejection of animals, and increased undesired alleles in animals used in industrial milk production. The degree of polymorphism detected in the *CSN3* gene confirmed that the PCR-SSCP technique was an efficient method for identifying allele variants, and that the sample size used (30 animals) was adequate for detecting the existing variability. The presence of the seven alleles in the *CSN3* gene demonstrates the extensive polymorphism found within the creole breeds. These alleles are associated with a range of habitats and management and production systems.

A high B allele frequency is desirable in industrialized milk production systems. The B allele is related to milk with high protein content, greater freezing-stability, higher cheese production (5-10%), shorter coagulation time, and more consistent curds (Van Eenennaam and Medrano, 1990). It is important to highlight that the B allele is predominant in the GCC breed (0.418 ± 0.20) and is above 55% in the CCC, the ROMO, and the CQT breeds. The low frequency of the B allele in the LUC breed is due to its genetic origins (40% HOLS, 30% Shorthorn, 30% HV) and its selection for achieving high milk yield. These traits are related to the *CSN3* A allele. However, the highest frequencies of the B allele were found in some meat producing breeds rather than milk or dual-purpose production breeds. This finding suggests a possible selection for cows based on maternal abilities and is expressed by the shorter standing time for calves raised on milk containing higher protein. For example, in the HV breed milk contained 3.37% protein and was superior to that of the Holstein breed milk. The composition was similar to the Ayrshire and Brown Swiss breeds (Hurtado, 1998).

Creole breeds on the American continent share a common origin with cattle introduced during the Spanish conquest era. The average value of the B allele in the Creole breeds was consistent with the values reported in America, the Caribbean, Cuba (Uffo *et al*., 2006), Brazil (Kemenes *et al.*, 1999; Lara *et al*., 2002), Perú (Veli *et al*., 2004), Argentina (Poli *et al*., 2002; Lirón *et al.,* 2002; Martínez *et al*., 2003), Bolivia (Lirón *et al.,* 2002), and Uruguay (Postiglioni *et al*., 2002). The frequency of the B allele in the Creole breeds was significantly higher ($p<0.05$) than that of the Holstein breed in Colombian-like conditions (López *et al.,* 1999; Solarte-Portilla *et al.,* 2009; Vivas *et al.,* 2008; Díaz *et al.,* 2006) and the specialized breeds (such as the Holstein, Guernsey, Milking Shorthorn, and Swedish Red) from other latitudes (Van-Eenennaam and Medrano, 1991; Beja-Pereira *et al.,* 2002; Barreras *et al.,* 2001; Tsiaras *et al.,* 2005; Caroli *et al.,* 2004; Van-Eenennaam and Medrano, 1991; Hallén *et al.,* 2009). Commercial breeds that are selected for higher production have a lower proportion of the B allele. This selection has drastically reduced the frequency of the alleles that are associated with milk quality. One method

to overcome this problem is to redirect selection schemes for specialized dairy breeds (Heck *et al.,* 2009). This study demonstrates that the GCC breed may be a sustainable alternative for improving milk quality in tropical climates.

The frequency of the A allele (0.39 ± 0.019) coincided with the reported range in the Bolivian Creole breeds (Lirón *et al*., 2002). However, the frequency was below that of the breeds from Argentina (Poli *et al.,* 2002; Lirón *et al*., 2002; Martínez *et al*., 2003), Perú (Veli *et al*., 2004), Cuba (Uffo *et al.,* 2006), Brazil (Kemenes *et al.*, 1999), Africa, and Italy (Ceriotti *et al.*, 2004).

The less common G, I, A_1, E, and N alleles represented 18% of the total frequency, which demonstrates that the Colombian Creole breeds maintain a high diversity in spite of their small population size. In the HV breed, Díaz *et al.,* (2006) found that the G, I, A_1, and E alleles represented 22% of the total frequency. These findings highlighted the importance of using the SSCP technique to correctly identify *CSN3* alleles. Although the A and B alleles were dominant in the GCC breed, the I allele was important in the SM breed (0.13 ± 0.044), and the G allele (listed as rare in *B. taurus* [Caroli *et al*., 2009]) was abundant in CAS (0.36), HV (0.23), and VEL (0.11) breeds. While there is no clear evidence regarding the impact of the G allele on milk, Prinzenberg *et al.* (1999) suggests that it has a negative effect on milk coagulation (similar to that of the A allele).

The A_1 allele was identified in seven of the twelve studied breeds and was most prominent in the CAS and BRAH breeds. Prinzenberg and Erhardt (1999) verified its specificity to zebuine cattle breeds. Nevertheless, Barrera *et al.* (2006) found no genetic proximity to the BRAH breed using the CAS breed microsatellite markers. The VEL breed (25% Red Brahman) showed very low A_1 allelic frequency. In addition, the E allele frequency was low in the CQT, CAS, SM, and VEL breeds (0.016). The E allele has been associated with poor coagulation (Ikonen *et al*., 1997; Hallén *et al*., 2007) and a higher casein proportion than that of the A allele (Heck *et al.,* 2009). The variant (N), which could not be identified using restriction enzymes or sequencing was only found in the ChS and VEL breeds. Therefore, this band pattern corresponds to an uncharacterized mutation.

The NA (4.60 ± 1.57) was noticeably higher than that reported for the majority of breeds from the Americas (Lirón *et al*., 2002; Postiglioni *et al.,* 2002; Martínez *et al*., 2003; Uffo *et al*., 2006, Lara *et al*., 2002; Veli *et al*., 2004; Kemenes *et al*., 1999). This trend probably occurred because these studies only identified two alleles. The expected heterozygosity (H_e=0.65) was in accordance with the ranges found in the creole breeds from the Americas (Lirón *et al*., 2002; Martínez *et al*., 2003) and the native Portuguese breeds (Beja-Pereira *et al*., 2002), but was considerably higher than the estimates made for the Colombian Holstein breeds (Solarte-Portilla *et al.,* 2009).

The high degree of polymorphism found in GCC reduces the effects of genetic drift and inbreeding (Harrison and Hastings, 1996), a process that tends to be common in small populations. This process brings about reduced biological performance, which is represented by lower survivorship, reduced reproductive performance, low growth rates, and difficulty in adapting to environmental changes (Saccheri *et al*., 1998). High variability in the Creole breeds from the Americas has been attributed to a series of causes, including low selection pressure, traditional management practices, introgressive hybridization with commercial breeds (Giovambattista *et al*., 2001; Lirón *et al*., 2006), demographic factors caused by European and African genes, and the breadth of geographical origin (Lirón *et al*., 2006).

The F_{ST} value and exact test used to analyze the degree of genetic differentiation among the studied Creole breeds showed significant differences between the GCC breeds. However, intrapopulational endogamy estimated by F_{is} was not significant.

In conclusion, a high degree of diversity for the *CSN3* locus was found in the Colombian Creole Breeds. In addition, a high CSN3 B allele frequency (related to milk quality) was found. A significant percentage was also found for other alleles, which emphasizes the importance of conserving the Creole breeds. These breeds contain a diverse

allele reservoir. Accordingly, they represent an opportunity for developing production alternatives that avoid genetic erosion.

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