Research article

Genetic polymorphism of beta-lactoglobulin and alpha-lactoalbumin in Colombian Creole cattle by PCR-SSCP

Polimorfismo genético de beta-lactoglobulina y alpha-lactoalbúmina en el ganado criollo colombiano, mediante PCR-SSCP

Jaime A. Rosero-Alpala[*], *Luz Ángela Álvarez-Franco*[†], and *Jaime Eduardo Muñoz-Flórez*[‡]

Department of Agricultural Sciences, Universidad Nacional de Colombia, A.A. 237. Palmira, Valle del Cauca, Colombia.
*Corresponding author: j_roseroa@yahoo.com.mx; †laalvarezf@unal.edu.co; ‡jemunozf@unal.edu.co

Rec.: 19.05.11 Acept.: 30.12.11

Abstract

The Colombian Creole Cattle has showed a disturbing decrease in population, from 23,415 individuals in 1999 to 20,102 in 2003. Despite that many efforts to recover the creole breeds have been done, its future conservation is unclear. Searching for economic desirable genes may contribute to its preservation and utilization as a genetic resource. Genes related with the improvement of milk proteins are considered as an economic important factor by the dairy industry. With the aim of characterizing beta-lactoglobulin (β-LG) and alpha-lactalbumin (α-LA) genes, 30 samples from each of the creole breeds (Blanco Orejinegro, Caqueteño, Casanareño, Costeño Con Cuernos, Chino Santandereano, Hartón del Valle, Romosinuano and Sanmartinero), two Colombian breeds (Lucerna and Velásquez) and two introduced breeds (Holstein and Brahman) were analyzed. A DNA fragment of 262 bp for β-LG and 166 for α-LA using PCR-SSCP were amplified and analyzed. The average frequencies for β-LG (A) and β-LG (B) were 0.46 ± 0.020 and 0.53 ± 0.020, respectively, and 0.35 ± 0.019 for α-LA (A) and 0.64 ± 0.019 for α-LA (B). The genetic diversity (He) average for β-LG was 0.498 and 0.455 for α-LA. Creole breeds represent a valuable genetic base as an alternative for breeding and improvement programs in dairy production herds in order to produce milk with desirable characteristics for the dairy industry.

Key words: α-LA, β-LG, Colombia, creole cattle, globulins, whey proteins.

Resumen

La población de ganado criollo colombiano ha venido presentando una inquietante disminución al pasar de 23,415 ejemplares en 1999 a 20,102 en 2003. A pesar de los esfuerzos por recuperar las razas criollas, el panorama para su conservación es incierto, por tanto la búsqueda de caracteres deseables puede contribuir a su valoración y conservación. Los genes relacionados con el mejoramiento de la calidad de la leche producida por estas razas se consideran de gran importancia en la industria láctea, por esta razón y con el objetivo de caracterizar los genes beta-lactoglobulina y alpha-lactoalbúmina se analizaron 30 muestras de sangre de cada una de las razas criollas (Blanco Orejinegro, Caqueteño, Casanareño, Costeño Con Cuernos, Chino Santandereano, Hartón del Valle, Romosinuano y Sanmartinero), dos razas sintéticas colombianas (Lucerna y Velásquez) y dos razas foráneas (Holstein y Brahman). Se amplificaron fragmentos de 262pb para beta-lactoglobulina (β-LG) y de 166 pb para alpha-lactoalbúmina (α-LA) que se genotipificaron mediante PCR-SSCP. El promedio de la frecuencia para β-LG A y β-LG B fue de 0.46 ± 0.020 y de 0.53 ± 0.020, respectivamente, y de 0.35 ± 0.019 para α-LA A y 0.64 ± 0.019 para α-LA B. El promedio de diversidad genética (He) para β-LG fue 0.498 y de 0.455 para α-LA. Los ganados criollos representan una base genética valiosa, como alternativa para mejorar genéticamente los hatos destinados a la producción de leche con mejores características en calidad para la industria láctea.

Palabras clave: α-LA, β-LG, bovinos criollos, Colombia, globulina, proteínas del suero.

Introduction

Colombia has a variety of breeds recognized as creole catle: Romosinuano (RS) and Costeño con Cuernos (CCC) in the Atlantic Coast, Blanco Orejinegro (BON) and Chino Santandereano (ChS) in the Andes or mountain regions, Hartón del Valle (HV) in the valley of Cauca river, Casanareño (CAS) and Sanmartinero (SM) in Orinoquia, el Caqueteño (CQT) in the Amazon and two breeds coming from creole cattle crosses: Velázquez (VEL) = Romosinuano 25%, Brahman Rojo 25% and Red Poll 50% in Caldas; and Lucerna (LUC) = Hartón del Valle 30%, Holstein 40% and Shorthorn 30%) in Valle del Cauca. The population of the Colombian creole cattle changed from 23,415 individuals in 1999 (Martínez, 1999) to 20,102 in 2003 without Casanareño population data (MADR-Asocriollo, 2003).

Foods from animal origin have an increasing demand, therefore, improvement of production systems is an urgent need including the dairy production systems. Cow milk is a complex mix of water, lactose, fat, proteins and other minor components. 95% of the total nitrogen is protean and is equivalent to 35 g of protein per kg of milk. 20% of the protein fraction corresponds to β-lactoglobulin and α-lactalbumin serum proteins.

β-Lactoglobulin (β-LG) represents about 50% of serum proteins and 12% of total protein from cow milk (Fox and McSweeney, 1998). The predominant variants in *Bos Taurus* breeds are: A (Gln 59, Asp64 and Val 118) and B (Gln 59, Gly 64 and Ala 118), however, other nine variants have been identified and evaluated (C, D, E, F, G, H, I, J, W) (Farrell *et al.,* 2004). β-LG variants are very important because of their association with k-casein, which is translated in total milk protein increment or reduction (Heck *et al.,* 2009). B allele of β-LG can be considered superior to A allele because its direct effect on mechanic resistance of gels, due to: (1) crosslink formations and aggregates implicated in serum proteins and products from curd hydrolysis and, (2) an increase in casein micelle size caused by β-LG B insertion in its surface, or to both cases; which improves the total solids proportions (Meza-Nieto *et al.,* 2007). β-LG allele A is associated with a lower proportion of β-LG (Wedholm *et al.,* 2006). β-Lactoglobulin (β-LG) BB genotype has been associated to a higher fat content, cheese yield and high casein porcentaje in milk (Caroli *et al.*, 2004). This is opposite to what is sugested for the β-LG AA genotype which is associated to a high total milk production (Ng-Kwai-Hang *et al.,* 1984).

α-Lactalbumin (α-LA) is a calcium metalloprotein which forms a complex with β-1,4 galactosyltransferase in the mammary epithelium to form, the lactose synthase enzyme which synthesize lactose in the secretory vesicle of the Golgi apparatus. Three variants (A, B and C) have been described, being A and B the most common ones. α-LA A variant has a Gln in the position 10, whereas α-LA B has an Arg (Farrell *et al.,* 2004). It has been demonstrated that Holstein cows with the A variant have high values for milk production, protein and fat, and, B variant cows show high protein and fat percentages (Bleck y Bremel, 1994). Likewise, a higher α-LA B proportion has been detected and, a higher milk production has been associated to the A allele (Heck *et al.*, 2009).

The main objective of the present work was to characterize milk production potential with desirable characteristics for industry in Colombian creole breeds, by means of frequency estimations, population parameters and differences between β-lactoglobulin and α-lactalbumin genes in ten Colombian creole breeds.

Materials and methods

354 blood samples were evaluated, they included eight creole breeds (30 individuals/breed), Blanco orejinegro (BON), Caqueteño (CQT), Casanareño (CAS), Costeño con cuernos (CCC), Chino Santandereano (ChS), Hartón del Valle (HV), Romosinuano (RS) and Sanmartinero (SM); two breeds coming from creole cattle crosses, Lucerna (LUC) and Velásquez (VEL); and two foreign breeds, Brahman (n = 24) and Holstein (n = 30), that where coming from different regions of Colombia and from the DNA bank of the Universidad Nacional de Colombia (Table 1).

DNA was isolated from 5ml of blood by using the ´Salting Out´ extraction protocol (Miller *et al.*, 1988). DNA quality was evalua-

ted in 0.8% agarose gels run on TBE 0.5X (0.045 M tris-borate, 0.001 M EDTA, pH 8.0) and dyed with ethidium bromide. 2 µl of DNA were mixed with 2 µl of bromophenol blue ((0.25% bromophenol blue and 30% glycerol). Samples were run in a horizontal electrophoresis chamber (BioRad wide mini sub-cell GT chamber) at 80V for 45. Gels were pictured under UV light with a digital camera (Kodak EDAS 290). DNA quantification was done by comparison with known concentrations of bacteriophage Lamba DNA.

For β-LG a 262 bp (Chromosome 1) fragment was amplified with the conditions described by Díaz *et al.* (2006). 50 ng/µl of DNA mixed with 3 µl of Tris-HCL (20mM) were used, it was denaturized at 95 °C on a thermocycler lowering down the temperature to 85 °C afterwards, then the PCR mix was added, this contained 100µM dNTPs, 0.75mM $MgCl_2$, 1 unit (U) Taq polymerase and 0.3 µM primer (each) (β-LG P3 5´ -GTC CTT GTG CTG GAC ACC GAC TAC A-3´ and β-LG P4 5´-CAG GAC ACC GGC TCC CGG TAT ATG A-3´). Denaturation was done at 97 °C for 35 cycles, each cycle was 94 °C for 1 min, , 60 °C for 1 min and 72 °C for 2 min with a final extension at 72 °C for 5 min, in a thermocycler PTC -100TM (MJ Research, Inc-USA).

For α-LA a 166 bp (Chromosome 5) was amplified with the conditions described by Díaz *et al.* (2006). 50 ng/µl of DNA were mix with a buffer solution containing 100µM dNTPs, 0.75mM $MgCl_2$, 1 unit (U) Taq polymerase and 0.1 µM primer (each) (sense, 5´ -CTC TTC CTG GAT GTA AGG CTT-3´ and antisense, 5´-AGC CTG GGT GGC ATG GAA TA-3´). Samples were subjected to an initial denaturation during 2 min. 35 cycles were one under the following conditions per cycle: 95 °C for 1 min, 55 °C for 1 min and 72 °C for 1 min and a final extension at 72 °C for 5 min.

Alleles were identified by PCR-SSCP (Single Strand Conformation Polymorphism). 2 µl of the PCR product were mixed with 8 µl of denaturizing buffer (xylene-cyanol 0.05%, bromophenol blue 0.05%, EDTA 5.5 mM pH 8.0). They were denaturized at 95 °C for 5 min and cooled down on ice for 2 min. Controls were samples of AA, AB and BB individuals previously genotyped by RFLPs by Díaz *et al.* (2006). Polyacrylamide gels were loaded Biometra® chamber 12 x 8 cm) (acrylamide ratio: N,N´-methylene bis acrylamide100:1) at 14% and 16% for β-LG and α-LA, respectively, 3.7% glycerol with TBE 0.5 X (0.045 M tris-borate, 0.001 M EDTA, pH 8.0). Electrophoresis was performed with a TBE 0.5 X for α–LA and 1X for β-LG. Gels were run at 160V for 10 h for β-LG and at 180V for 4 h for α-LA, under constant temperature of 12 °C.

Parameters estimated were: Allele frequen-

Table 1. Sampling size and locations of creole, Colombian and foreign cattle breeds.

Breed	**No.**	**Location**
		Town (Department)
Blanco Orejinegro	30	Popayán (Cauca)
Caqueteño	30	Florencia y Morelia (Caquetá)
Casanareño	30	Arauca (Arauca)
Costeño con cuernos	30	Campeche (Atlántico)
Chino Santandereano	30	San Gil y San Alberto (Santander)
Hartón del Valle	30	Tuluá, Jamundí, Palmira (Valle del Cauca) [a]
Lucerna	30	Valle de Cauca
Romosinuano	30	Sincerin (Bolívar)
Sanmartinero	30	San Martín (Meta)
Velásquez	30	La Dorada (Caldas)
Brahman	24	Jamundí (Valle del Cauca) [a]
Holstein	30	Yotoco, Candelaria (Valle del Cauca)[a]

a. DNA bank, Universidad Nacional de Colombia.

cies, observed heterozyigosity (Ho), expected heterozygosity (He), inbreeding coefficient (F_{IS}), Hardy-Weinberg equilibrium (HWE) and genetic differentiation coefficient (F_{ST}), using Arlequin software (Integrated Software Package for Population Genetics Data Analysis) version 3.1 (Excoffier *et al.*, 2006).

Results and discussion

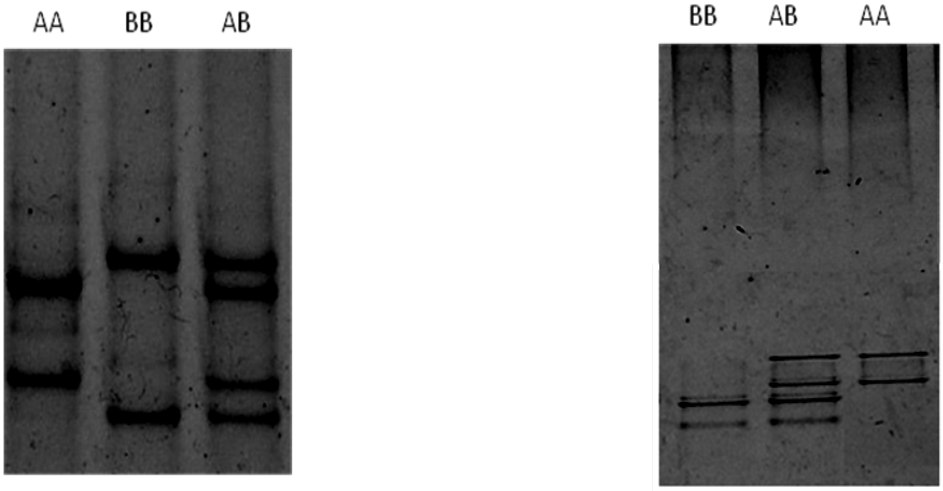
Two bands patterns were detected for α-LA and β-LG by PCR-SSCP (Picture 1). Allele frequencies for β-LG and α-LA genes in the different creole and foreign breeds are presented in Table 2. Only β-LG A and B variants were detected by PCR-SSCP.

Frequency for β-LG B (0.53 ± 0.02) was higher than the one for β-LG A (0.46 ± 0.02). β-LG B variant – that improves total solids proportions (Meza-Nieto *et al.*, 2007)- was found in high frequency in CQT, LUC, HV and ChS breeds and in lower proportion in the creole breeds BON, CAS, SM and VEL. Average allele frequency for β-LG B in creole breeds was higher than in Holstein.

As in this study, in most of tropical American creole breeds there is a higher frequency of β-LG B allele of interest than β-LG A allele (Postiglioni *et al.*, 2002; Lirón *et al.*, 2002; Poli *et al.*, 2002; Rincón *et al.*, 2006). Frequency estimates for Colombian creole breeds are in the range described for commercial breeds of US (Van-Eenennaam and Medrano, 1991), Italy (Caroli *et al.,* 2004), Greece (Tsiaras *et al.*, 2005) and Portugal (Beja-Pereira *et al.,* 2002). A higher frequency for β-LG B is desirable since it correlates with proteins of better quality in milk and improves aggregates formation in the curd at industrial level (Meza-Nieto *et al.*, 2007). The high proportion of the β-LG B allele of interest found in creole breeds represents an alternative for use in double purpose systems focused on cheese production. This due to the fact that β-LG B allele have been associated with higher fat and casein content in milk.

β-LG A allele found in high frequencies in the Colombian breeds CAS, VEL, SM and BON was similar to what is reported for the Brazilian breed Caracú (0.57) (Kemenes *et al.,* 1999) and the commercial breed Holstein (0.58) (Heck *et al.,* 2009).

For α-LA there was a higher frequency of the B variant (0.64 ± 0.01) than of the A variant (0.35 ± 0.01). The allele of interest α-LA B was found in high frequency in all the breeds except for BON and CQT. α-LA is considered important because it forms part of the complex β-1,4 galactosyl transferase, which is responsible of lactose synthase formation for lactose synthesis. Bleck and Bremel (1994) demonstrated that Holstein cows with the A variant had higher protein and milk production levels, in comparison with B allele cows



Picture 1. Run patterns for two allelic variations on a region of the β-LG (left) and α-LA (right) genes by PCR-SSCP.

Table 2. Genetic frequencies and standard deviations of β-LG alellic variants and α-LA in Colombian creole breeds and in two foreign breeds.

Breeds	**β-LG**		**α–LA**	
	A	**B**	**A**	**B**
BON	0.51±0.065	0.48±0.065	0.55±0.065	0.45±0.064
CQT	0.28±0.058	0.71±0.058	0.50±0.065	0.50 ±0.065
CAS	0.65± 0.062	0.31± 0.060	0.33±0.061	0.66±0.061
ChS	0.36±0.062	0.63±0.062	0.31±0.060	0.68 ±0.062
CCC	0.46±0.064	0.53±0.064	0.30±0.059	0.70±0.059
HV	0.31±0.060	0.65±0.062	0.15±0.035	0.85±0.046
LUC	0.35±0.062	0.65±0.062	0.21±0.053	0.78±0.053
RS	0.43±0.064	0.56 ±0.064	0.30±0.059	0.70±0.059
SM	0.63±0.062	0.36±0.062	0.43±0.064	0.56±0.064
VEL	0.63±0.062	0.36±0.062	0.43±0.064	0.56±0.064
Average	**0.46±0.020**	**0.53±0.020**	**0.35± 0.019**	**0.64±0.019**
Brahman	0.28±0.069	0.67±0.069	0.00±0.00	1.0± 0.0
Holstein	0.60±0.063	0.40±0.063	0.53 ± 0.064	0.46±0.064

that show higher protein and fat percentage. α-LA gen has not been widely used to evaluate creole or commercial breeds in America as it is done with the k-casein (k-CN) or β-LG genes. These are the first findings showing α-LA gen frequency in Colombian creole breeds. The higher frequency of α-LA B allele in Colombian creole breeds agrees with the findings on creole breeds in Uruguay (Postiglioni *et al.*, 2002; Rincón et al., 2006) and the Colombian creole breed Hartón del Valle (Díaz *et al.,* 2006) being superior to what has been reported for Cubana and Siboney breeds (Uffo *et al.,* 2006). Although Uffo *et al.* (2006) suggested the presence of the α-LA A allele as introgression indicator in zebu breeds *Bos indicus*), in this study the A allele was found in all Colombian creole breeds as well as in Holstein, and exceptionally, it was not detected un Brahman.

Expected heterozygosity estimates (He), the Hardy-Weinberg equilibrium test (HWE) and inbreeding coefficient (F_{IS}) can be found on Table 3. In creole breeds genetic diversity values (He) for β-LG varied between 0.41 and 0.50, with an average of 0.49. BON and CCC showed the highest genetic diversity values. For CQT, ChS, HV and LUC breeds there was no HWE. F_{IS} was significant only for LUC. He value for α-LA was between 0.16 and 0.5, with an average of 0.45. The highest He values were found in BON, CQT, SM and VEL. HWE was not found in BON, CQT, RS and Holstein and, the inbreeding coefficient was significant for LUC and RS (Table 3). The index for genetic diversity was highly significant between the studied breeds. (F_{ST} = 0.077; P < 0.01).

He determination (0.498 ± 0.02) for β-LG in the Colombian creole breeds were in the range described for South American creole breeds (0.267 - 0.508) (Lirón *et al.*, 2002; Rincón *et al.*, 2006), for Portuguese breeds (0.27 - 0.5) (Beja-Pereira *et al.*, 2002), and for the Colombian breed Hartón del Valle (Díaz *et al.,* 2006). HWE deviations in the CQT and ChS breeds could be associated with their small population size and, to the preference of some males in the herd where they stay for different generations.

He average value for the α-LA gen in the Colombian creole cattle the high He values obtained for most of the breeds are higher than the ones reported by Díaz *et al.* (2006). HWE deviations in the CQT creole breed could

Table 3. Estimated values for expected heterozygosity (He) and inbreeding coefficient (F_{IS}) for β-LG and α-LA in Colombian creole and foreign breeds (Holstein y Brahman).

	He		F_{IS}	
Breed	**β-LG**	**α-LA**	**β-LG**	**α-LA**
BON	0.507	0.50**	-0.25	-0.812
CQT	0.413*	0.50**	0.43	-1.0
CAS	0.448	0.452	0.15	0.265
CCC	0.506	0.427	0.21	0.06
ChS	0.472*	0.448	0.58	-0.31
HV	0.448*	0.165	0.46	-0.08
LUC	0.462*	0.345	0.50*	0.32*
RS	0.499	0.427*	0.33	0.69*
SM	0.472	0.499	0.29	0.201
VEL	0.472	0.499	0.24	0.066
Average	**0.498****	**0.455**	**0.33**	**-0.019**
Brahman	0.426*	0.000	0.69	
Holstein	0.481	0.506**	0.04	-0.87

F_{ST} 0.077 (P < 0.01).
HWE according to the exact test used by the Arlequín 3.1 statistical software (Excoffier *et al.*, 2006) using the Markoviana chain with predicted length =100000; No. of memorizations = 1000.

be coupled to lower effective population size in this breed; whereas in BON and RS creole breeds could be associated to an excess or deficiency of heterozygotes and, for Holstein could be related to selective pressure. Although, for some creole breeds HWE for β-LG and α-LA genes was found, it is not possible to ensure the allele frequency stability since in some breeds there is a low effective size which causes a sampling error, as it is stated by Caujapé-Castells (2006).

The present study demonstrate that high genetic diversity is supported not only by the presence of at least two alleles in the evaluated genes, but also by the high He values found in most of the Colombian creole breeds, despite of the small populations and limited number of reproductive males, which could increment endogamy levels.

The genetic differentiation value (F_{ST} = 0.077) in the Colombian creole cattle, illustrates the importance of local breeds evaluations, being this parameter useful in zoogenetic resources conservation and management, because they give an indication of the origin and genetic diversity magnitude among them.

Conclusions

- The high frequency for the β-LG B allele and α-LA demonstrates the Colombian creole cattle value for milk production with desirable characteristics for industry. The high genetic diversity values indicate that the Colombian creole cattle is a resource with high genetic diversity in milk proteins.
- CQT, ChS, HV and LUC creole breeds are potential candidates for genetic breeding programs aiming to increase milk quality levels.

Acknowledgements

To the Research Program of the Universidad Nacional de Colombia – Palmira (DIPAL) for financing (Proyecto 20101100727). To the creole breeds breeders Marino Valderrama and Eduaime Cárdenas (Hartón del Valle), Juan Manuel Gonzales and Julia Arias (BON), Rafael Torrijos and Francisco Ramón (CQT), Germán Martínez (SM), Luis Fernando Cala (ChS), Arturo Cabrera (CCC), Rodrigo Salas (ROMO), José Antonio Velásquez (VEL), Pablo Canay and Arcesio Salamanca (CAS).

References

Beja-Pereira, A.; Erhardt, G.; Matos, C.; Gama, L; and Ferrand, N. 2002. Evidence for a geografical cline of casein haplotypes in portiuguese cattle breeds. Anim. Gen. 33: 295 - 300.

Bleck, G. T. and Bremel, R. D. 1994. Variation in expression of a bovine α-lactalbumin transgene in milk of transgenic mice. J. Dairy Sci. 77(7):1897 - 1904.

Caroli, A.; Chessa, S.; Bolla, P.; Budelli, E.; and Gandini, G. C. 2004. Genetic structure of milk protein polymorphism and effects on milk production traits in local dairy cattle. J. Anim. Breed. Genet. 121(2):119 - 127.

Caujapé-Castells, J. 2006. Brújula para botánicos desorientados en la genética de poblaciones. Exegen Ediciones. Las Palmas de Gran Canaria. España. 132 p.

Díaz, H. A.; Alvarez, L. A.; Muñoz J. E.; Posso A.; and Sanabria, H. L. 2006. Genetic variability of milk proteins (k-Casein, B-Lactoglobulin and a-Lactoalbumin) in Hartón Del Valle Creole cattle. Proceedings of the 30th International Conference on Animal Genetics. Porto Seguro, Brazil. Belo Horizonte, Brazil: CBRA, ISBN 85-85584-03-3 (CD); 85-85584-02-5 (site www.cbra.org.br).

Excoffier, L.; Laval, G.; and Schneider, S. 2006. Arlequin ver 3.1: An Integrated software package for population genetics data analysis. Manual Arlequin. 145 p.

Farrell, H. M. Jr.; Jimenez-Flores, R.; Bleck, G. T.; Brown, E. M.; Butler, J. E.; Creamer, L. K.; Hicks, C. L.; Hollar, C. M.; Ng-Kwai-Hang, K. F.; and Swaisgood, H. E. 2004. Nomenclature of the proteins of cows' milk. Sixth revision. J Dairy Sci. 87 (6):1641 - 1674.

Fox, P. F. and McSweeney, P. L. 1998. Dairy chemistry and biochemistry. Londres. Blackie Academic & Professional. 478 p.

Heck, J. M. L.; Schennink, A.; van Valenberg, H. J. F.; Bovenhuis H.; Visker, M. H. P. W.; van Arendonk, J. A. M.; and van Hooijdonk, A. C. M. 2009. Effects of milk protein variants on the protein composition of bovine milk. J Dairy Sci. 92(3):1192 - 1202.

Kemenes, P. A.; De Almeida Regitano, L. C.; De Magalhaes Rosa, A. J.; Packer, I. U.; Razook, A. G.; Andrade de Figueiredo, L.; Silva, N. A.; Etchegaray, M. A. L.; and Lehmann Coutinho, L. 1999. κ-casein; β-lactoglobulin and growth hormone allele frequencies and genetic distance in Nelore, Gyr, Guzerá, Caracu, Charolais, Canchim and Santa Gertrudis Cattle. Genet. Mol. Biol. 22(4):539 - 541.

Lirón, J. P.; Ripoli, M. V.; De Luca, J. C.; Peral-García, P.; and Giovambattista, G. 2002. Analysis of genetic diversity and population structure in Argentine and Bolivian creole cattle using five loci related to milk production. Genet. Mol. Biol. 25(4):413 - 419.

MADR-Asocriollo. 2003. Población por grupos raciales. En: Razas criollas y colombianas puras. MADR y Asocriollo (Asociación de Criadores de Ganado Criollo y Colombiano). Memoria Convenio 135-01.

Martínez, G. 1999. Memorias del Seminario: Censo y Caracterización de los Sistemas de Producción del Ganado Criollo y Colombiano. Fedegan, ICA, Pronatta y Asobon. Santafé de Bogotá. 158 p.

Meza-Nieto, M. A.; Vallejo-Cordoba, B.; González-Córdova, A. F.; Félix, L.; and Goycochea, F. M. 2007. Effect of β-lactoglobulin A and B whey protein variants on the rennet-induced gelation of skim milk gels in a model reconstituted skim milk system. J Dairy Sci. 90(2):582 - 593.

Miller, S. A.; Dykes, D. D.; and Polesky, H. F. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic. Acids. Res. 16(3):1215.

Ng-Kwai-Hang, K. F.; Hayes J. F.; Moxley J. E., and Monardes H.G. 1984. Association of genetic variants of casein and milk serum proteins with milk, fat, and protein production by dairy cattle. J. Dairy Sci. 67:835-840.

Poli, M. A.; Holgado, F.; and Rabasa, A. E. 2002. Frecuencias genotípicas y alélicas de los genes de caseína k y la lactoglobulina B en un rodeo de bovinos criollos en Argentina. En: III Simposio Iberoamericano sobre la conservación de los recursos zoogenéticos locales y el desarrollo rural, 25-27 nov, 2002. Montevideo, Uruguay, UDELAR. Pp. 12.

Postiglioni, A.; Rincón, G.; Kelly, L.; Llambí S.; Fernández, G.; D'Angelo, M.; Gagliardi, G.; Trujillo, J.; de Bethencourt, M.; Guevara, K.; Castellano, A.; and Arruga, M. V. 2002. Biodiversidad genética en bovinos criollos del Uruguay. Análisis con marcadores moleculares. *Arch. Zootec.* 51: 195-202.

Rincón, G.; Armstrong, E.; and Postiglioni, A. 2006. Analysis of the population structure of Uruguayan Creole cattle as inferred from milk major gene polymorphisms. *Genet. Mol. Biol.* 29 (3): 491-495.

Tsiaras, A. M.; Bargouli, G. G.; Banos, G.; and Boscos C. M. 2005. Effect of kappa-casein and beta-lactoglobulin loci on milk production traits and

reproductive performance of Holstein cows. *J. Dairy Sci.* 88 (1): 327-334.

Uffo, O.; Martín-Burriel, I.; Martínez, S.; Ronda, R.; Osta, R.; Rodellar, C.; and Zaragoza, P. 2006. Caracterización genética de seis proteínas lácteas en tres razas bovinas cubanas. *Animal Genetic Resources Information* 39: 15-24.

Van-Eenennaam, A.; Medrano, J. F. 1991. Milk protein polymorphisms in California Dairy Cattle. *J Dairy Sci.* 74 (5): 1730-1742.

Wedholm, A.; Larsen, L. B.; Lindmark-Mànsson, H.; Karlsson, A. H.; and Andrén, A. 2006. Effect of protein composition on the cheese-making properties of milk from individual dairy cows. *J Dairy Sci.* 89 (9): 3296-3305.