rccp Revista Colombiana de Ciencias Pecuarias

Genetic structure analysis of a Holstein cow population in Colombia¤

Análisis de la estructura genética de una población de bovinos Holstein en Colombia

Estrutura genética de uma população de bovinos da raça Holandesa na Colômbia

Julián Echeverri[1]*, Zoot, MSc, PhD; Clara I. Saldamando[2], Biol, PhD; Albeiro López-Herrera[1], Zoot, MV, MSc, PhD.

[1]*BIOGEM Research Group, Facultad de Ciencias Agrarias, Universidad Nacional de Colombia, Medellín, Colombia.*
[2]*Facultad de Ciencias, Universidad Nacional de Colombia, Medellín, Colombia.*

(Received: March 20, 2013; accepted: May 16, 2014)

Summary

Background: genetic improvement in Colombia is limited by the lack of phenotypical and genealogical information. Additionally, molecular markers are important tools for studying the genetic structure of populations. **Objective:** to determine the structure of a population of Holstein cows in Antioquia, Colombia. **Methods:** a population of 1,800 animals distributed over 178 herds in 11 municipalities of Antioquia (Colombia) was genotyped using PCR-RFLP for gene polymorphisms of bovine growth hormone, kappa casein, prolactin, and BoLA DRB3.2. Population structure parameters, such as all Wright´s F-statistics, were calculated and Hardy Weinberg equilibrium was determined. Analysis of molecular variance was carried out and Nei distances were estimated to determine population differentiation. **Results:** the total population was in Hardy Weinberg equilibrium for the four genes; however, kappa casein gene was in disequilibrium in some of the populations. Genetic structure of populations shows little genetic differentiation between them. Furthermore, a trend for outcrossing was found in most populations; this may be due to incorporation of imported semen into Colombia's dairy farms. The most genetically distant populations were in Marinilla and Rionegro municipalities due to their technological level and geographic location, which places them outside the scope of artificial insemination programs with foreign semen. **Conclusions:** the genetic similitude between populations of Holstein cows in Antioquia is due to their geographic proximity; therefore, their management of genetic conditions is very similar.

Keywords: *animal production, molecular markers, population genetics, SNP.*

Resumen

Antecedentes: el mejoramiento genético en Colombia está limitado por la deficiencia en la información fenotípica y genealógica existente. Los marcadores moleculares son una importante herramienta para el

¤ To cite this article: Echeverri J, Saldamando CI, López-Herrera A. Genetic structure analysis of a Holstein cow population in Colombia. Rev Colomb Cienc Pecu 2015; 28:54-63.

* Corresponding author: Julián Echeverri. Facultad de Ciencias Agrarias, Universidad Nacional de Colombia. Calle 59A No 63-20 Medellín, Colombia-Núcleo El Volador, Bloque 50, Oficina 310. E-mail: jjecheve@unal.edu.co

estudio de la estructura genética de poblaciones. **Objetivo:** el objetivo de esta investigación fue estudiar la estructura genética de una población de vacas Holstein del departamento de Antioquia, Colombia. **Métodos:** una población de 1.800 animales ubicados en 178 hatos de 11 Municipios del Departamento de Antioquia (Colombia) fueron genotipificados mediante la técnica de PCR-RFLP, para los polimorfismos de los genes de la hormona de crecimiento bovino, kappa caseína, Prolactina y BoLA DRB3.2. Los parámetros de estructura poblacional, como los estadísticos F-Wright, fueron calculados y el equilibrio de Hardy Weinberg determinado. Un análisis de varianza molecular fue llevado a cabo y las distancias de Nei estimadas para determinar la diferenciación poblacional. **Resultados:** se encontró, que la población total se encuentra en equilibrio de Hardy Weinberg para los cuatro genes, sin embargo el gen de la kappa caseina se encuentra en desequilibrio en algunas poblaciones particulares. Los resultados mostraron que la población tiene estructura genética con una pequeña diferenciación genética entre ellas. Además, se encontró tendencia a la exogamia en la mayoría de las poblaciones, producto quizás de la incorporación de semen importado en las ganaderías de leche del país. Las poblaciones más distanciadas genéticamente son Marinilla y Rionegro, poblaciones que por su nivel tecnológico y por su localización geográfica, han estado al margen de la influencia de los programas de inseminación artificial con semen extranjero, implementados en algunas de las demás subpoblaciones involucradas en la investigación. **Conclusiones:** este estudio encontró similitud entre las poblaciones de vacas Holstein en Antioquia, la cual es debida a su proximidad geográfica; por esto el manejo de las condiciones genéticas es muy similar.

Palabras clave: *genética de poblaciones, marcadores moleculares, producción animal, SNP.*

Resumo

Antecedentes: o melhoramento genético na Colômbia é limitado pela deficiência de informação fenotípica e genealógica. Os marcadores moleculares são uma ferramenta importante para o estudo da estrutura genética de populações. **Objetivo:** o estudar a estrutura genética de uma população de vacas da raça Holandesa do departamento de Antioquia. **Métodos:** uma população de 1.800 animais em 178 rebanhos localizados em 11 municípios de Antioquia foram genotipados por PCR-RFLP, para os polimorfismos dos genes do hormônio de crescimento bovino, kappa caseína, prolactina e BoLA DRB3.2. Parâmetros de estrutura da população, como F-Wright estatísticas foram calculados e o estado de Hardy Weinberg determinado. Uma análise de variância molecular foi realizada, e as distâncias de Nei estimadas para determinar diferenciação da população. **Resultados:** verificou-se que a população total se encontra em equilíbrio de Hardy Weinberg para os quatro genes, além de encontrar um desvio do equilíbrio, no caso de kappa caseína gene em algumas populações específicas. Os resultados mostraram que a estrutura genética da população tem encontrado uma pequena diferenciação genética entre elas. Além disso, a tendência para a exogamia foi encontrada na maioria das populações, talvez seja consequência da incorporação de sêmen importado para o gado de leite no país. As populações geneticamente mais distantes foram Marinilla e Rionegro, populações que por sua localização geográfica e seu nível tecnológico, têm sido deixadas fora da influência de programas de inseminação artificial com o sêmen estrangeiro, estes programas têm sido utilizados em algumas das outras subpopulações envolvidas na pesquisa. **Conclusões:** este estudo constatou que a semelhança genética entre as populações de vacas holandesas em Antioquia é devida à sua proximidade geográfica, portanto, suas condições de manejo genéticas são muito semelhantes.

Palavras chave: *genética de populações, marcadores moleculares, produção animal, SNP.*

Introduction

Genetic improvement has recently slowed down in Colombia due to the lack of phenotype and genotype information. The understanding of a population's genetic structure has improved with the recent use of molecular single nucleotide polymorphism (SNP) markers and the development of molecular techniques.

Dairy yield involves many genes; numerous interactions between them are responsible for the phenotypic expression of each trait. Some of these genes have polymorphisms, which make them useful to understand the genetic structure of a population. Some include bovine growth hormone (BGH), prolactin (PRL), bovine lymphocyte antigen (BoLA), and the kappa casein (KC) gene.

Genetic structure of a population is characterized by the number of subpopulations within it, the frequencies of different genetic variants (alleles) in each subpopulation, and the degree of genetic isolation of the subpopulations (Haldane, 1954).

Several polymorphisms have been reported for BGH (Chikuni *et al.,* 1994**;** Yao *et al.,* 1996; Dybus, 2002; Ge *et al.,* 2003; Zakizadeh, 2006; Echeverri *et al.,* 2010a), PRL (Lewin *et al.,* 1992; Chrenek *et al.,* 1998; Wodjak *et al.,* 2008; Aijun *et al.,* 2010; Echeverri *et al.,* 2010b), KC (MacKinlay and Wake, 1964; Neelin, 1964;Barany *et al.,* 1997; Barroso *et al.,* 1999; Penket *et al.,* 1999; Ju *et al.,* 2008), and BoLA genes (Sigurdardotir *et al.,* 1991; Dietz *et al.,* 1997a; Dietz *et al.,* 1997b; Zambrano *et al.,* 2010). The SNP markers are located in different positions.

Polymorphism detected in these genes and their apparent importance in productive, reproductive, and health processes in cattle make it necessary to know their genetic structure in Colombian cattle populations.

The use of foreign semen in artificial insemination programs is common in Colombia, specifically in the subpopulations of Antioquia. However, improvement objectives are not necessarily the same for all subpopulations. This is perhaps the cause of the genetic diversity observed in these sub-regions. This study is the first step towards understanding the genetic structure of populations for these genes and their possible association with traits of economic importance.

The main goal of this study was to determine the parameters of population structure for some BGH, PRL, KC, and BoLA DRB3.2 gene polymorphisms in a population of Holstein cows located in Antioquia, Colombia.

Materials and methods

Location

This study evaluated 178 herds distributed over 11 municipalities in Antioquia. Herds were located in the two main dairy regions: Northern and Eastern Antioquia (both regions are 60 km apart from each other). Genetic evaluation was based on the genotyping of 1366, 1633, 1462, and 281 animals for gene polymorphisms for BGH, KC, PRL, and BoLA, respectively. All municipalities and genes were included in some analyses. Municipalities with few individuals for BoLA DRB3.2 gene were removed.

Genotyping

DNA extraction was carried out using the salting out method (Sambrook, *et al.,* 1989). Amplification and genotyping of specific regions of BGH, PRL, and KC genes used the methods described by Brym (2004), Dybus (2002), and Medrano *et al*. (1990), respectively. Amplification of the specific region for BoLA DRB3.2 gene used the methodology developed by Van Eijk *et al*. (1992).

Amplification and digestion of specific regions of KC, BGH, and PRL genes

Amplification of bovine KC gene used PCR, generating an amplified product of 344 bp which, after digestion with HinfI restriction endonuclease, produced three restriction patterns that generated three genotypes: AA, AB, and BB. The BGH gene generated an amplified 329 bp product, which, after digestion with MspI restriction endonuclease, produced three different restriction patterns that generated three genotypes: +/+, +/-, and -/-.

The amplified 296 bp product, which corresponds to the region of bovine PRL gene, was digested with RsaI enzyme, producing three different restriction patterns that generated three genotypes: AA, AB, and BB.

The specific region of BoLA gene was amplified with two PCR rounds. This yielded a 284 bp amplification product, which was digested with RsaI, HaeIII, and BstyI restriction enzyme producing 106 genotypes resulting from the combination of 32 alleles. These alleles were identified as described by Van Eijk *et al*. (1992), Gelhaus *et al*. (1995), and Maillard *et al*. (1999).

Population analysis

Allele and genotype frequencies were calculated for the total population and each municipality. Then,

comparisons were made regarding differences between each population. The methodology described by Hartl (2000) was used for this purpose. The Hardy Weinberg equilibrium analysis of genes was carried out independently taking into account the procedure described by Guo and Thompson (1992). The Hardy Weinberg equilibrium evaluation was performed using the Genepop software (Raymond and Rousset, 1995). Likewise, the genetic structure analysis of the population was carried out with an analysis of molecular variance (AMOVA) and FIS (inbreeding coefficient of an individual [I] relative to the subpopulation [S]), FIT (inbreeding coefficient of an individual [I] relative to the total [T] population), and a paired FST (effect of subpopulations [S] compared to the total population [T] estimate) for the 11 municipalities of Antioquia. F-statistics can also be used to measure correlation between genes drawn at different levels of a (hierarchically) subdivided population. Several evolutionary processes, such as mutation, migration, inbreeding, natural selection, or the Wahlund effect influence this correlation, but it was originally designed to measure the amount of allelic fixation due to genetic drift.

The Arlequín software was used for the estimation of AMOVA and paired distances (Scheneider *et al*., 2000). After the analysis of molecular variance, the Nei genetic distances between subpopulations were estimated, whilst separately taking into account every molecular marker. These distances were obtained using the Genealex (Peakall and Smouse, 2006) software and the UPGMA trees constructed with the MEGA 5 software (Tamura *et al.,* 2011).

Results

Allele and genotype frequencies of KC, BGH, PRL, and BoLA DRB3.2 genes

For the KC gene, the frequencies for alleles A and B in the entire population were 0.65 and 0.35, respectively. These trends were generalized in all the municipalities involved in the study, except Marinilla and Rionegro, where allele B had a higher frequency, namely 0.76 and 0.85, respectively.

The genotype frequencies for the KC gene were 0.45, 0.41, and 0.14 for genotypes AA, AB, and BB, respectively. Genotypes AA and AB had the highest frequencies and were similar in almost all municipalities except Marinilla and Rionegro, where genotype BB was the most frequent. Allele frequencies for BGH gene were 0.87 and 0.13 for alleles (+) and (-), respectively. These frequencies were similar in all municipalities involved in the study. However, Marinilla, Rionegro, and San Pedro de los Milagros municipalities had the highest frequencies for allele (-), with 0.22, 0.15, and 0.14, respectively. Municipalities with the lowest frequencies for this allele were Envigado and Don Matías, with 0.03 and 0.04, respectively.

The most frequent genotype was (+/+), with 0.76, compared to the (+/-) and (-/-) genotypes, whose frequencies were 0.22 and 0.013, respectively. Envigado had the highest frequency of individuals with genotype (+/+), which amounted to 0.92. The lowest frequency for this genotype was found in Marinilla (0.62).

For the PRL gene, the most frequent allele was allele A, with a frequency of 0.83; allele B had a frequency of 0.17. Municipalities with a higher frequency of allele A were San Pedro de los Milagros and La Unión, with a value of 0.88. The lowest frequency for this allele was found in Marinilla, with a value of 0.56.

The three genotypes found for the PRL gene were AA, AB, and BB; they had a frequency of 0.70, 0.27, and 0.03, respectively. These frequencies were very similar in all municipalities. However, Rionegro, and Marinilla had the lowest frequencies of the most prevalent genotype, with values of 0.58 and 0.38, respectively. The highest frequencies for AA were found in La Unión and San Pedro de los Milagros, with a value of 0.77.

Polymorphism of exon 3 of BoLA turned out to be the most polymorphic one of the four genes examined in this study. This was expected due to the fact that a hyper variable antigen-binding region was amplified. Thirty-three alleles, which generated 106 different genotypes, were found in the entire population, although only 281 animals were included for the study of this gene.

The most frequent were alleles 8 and 16, with frequencies of 0.15 and 0.14, respectively. Not all the alleles were found in all the populations; this was to be expected given the low number of individuals in this analysis and the high level of polymorphism in this gene.

The five most frequent genotypes in the studied population were genotypes 8/16, 8/23, 8/24, 16/22, and 10/15, with frequencies of 0.060, 0.053, 0.042, 0.039, and 0.035, respectively.

The lowest frequency was found for 51 genotypes. They were only found in one of the individuals of the total population. This yielded a frequency of 0.36 for these genotypes.

Determination of the Hardy Weinberg equilibrium

The population had equilibrium for genes PRL and BGH upon including all the animals in the analysis for the Hardy Weinberg equilibrium. Despite this, there was a significant difference between the expected and observed heterozygosity for the KC and BoLA genes. Variations were found in the genes in the Hardy Weinberg equilibrium when the population was divided into eleven subpopulations or municipalities. This indicates that somehow the objectives of artificial selection or the action of natural selection processes have been different in these municipalities, and changes in gene frequencies have not been consistent in all subpopulations. The PRL and BGH genes were in equilibrium in all subpopulations. The KC and BoLA genes were in disequilibrium in some subpopulations.

Table 1 shows the p-values for the comparison of expected and observed heterozygosity in the total population and in every municipality or subpopulation. It also indicates the state of equilibrium for each gene.

The Hardy Weinberg equilibrium results show that populations are in equilibrium, except for Rionegro and San Pedro. Therefore, there is apparently no strong selection effect leading to allele fixation in the other subpopulations. However, the population size used for this analysis in San Pedro as well as the total population results suggest the existence of a selection effect for KC and BoLA DRB3.2 genes. Results show that some of these markers act as neutral markers. Thus, an analysis of the population structure based upon the genes would be more reliable.

Linkage disequilibrium

Several authors have associated the genes studied in this project with economically important traits for dairy cattle, such as dairy yield, dairy component production, and resistance to diseases such as mastitis (Falaki *et al.,* 1996; Dybus, 2002; Echeverri *et al.,*

Table 1. Hardy Weinberg equilibrium for KC, BGH, PRL, and BoLA genes for the total population and 11 subpopulations of Antioquia.

Municipality	Total		Medellín		Belmira		Bello		Don Matías		Entrerríos	
Locus	N*	P**	N	P	N	P	N	P	N	P	N	P
PRL	1453	0.85	93	0.45	133	0.56	129	0.56	33	1.00	337	0.69
KC	1614	0.00	99	0.13	154	0.09	138	0.80	37	0.48	356	0.25
BGH	1357	0.71	95	0.59	71	1.00	113	0.29	36	1.00	317	0.50
BoLA	267	0.00	17	0.14	1	1.00	36	0.00	9	1.00	45	0.09
	Envigado		**La Unión**		**Rionegro**		**San Pedro**		**Santa Rosa**		**Marinilla**	
Locus	N	P	N	P	N	P	N	P	N	P	N	P
PRL	15	0.05	120	1.00	110	0.78	441	1.00	16	0.50	26	0.13
K-Casein	15	0.23	150	0.16	123	0.00	490	1.00	23	0.09	29	0.42
BGH	16	1.00	126	0.66	121	0.47	419	1.00	14	1.00	29	0.59
BoLA			12	0.81	36	0.01	109	0.00	2	1.00		

* Number of animals; ** p-value for the difference between expected and observed heterozygosity.

2010a; Echeverri *et al.,* 2010b). These characteristics have also been extensively studied with genetic correlations indicating the existence of pleiotropic genes coding for two or more of these traits, affecting them in a similar or different manner, depending on the case. A linkage analysis was conducted to determine whether the genes involved in the phenotypic variation of these characteristics are in linkage equilibrium. This analysis was conducted both in a general and particular manner for each subpopulation. All four genes were in linkage disequilibrium when the analysis included the total population, thus indicating that genes tend to be associated with each other more often than one would expect if they were independent. This is important because accurately determining the type of association between genes enables researchers to select for some genes whilst only knowing the genotype of another gene with which the former is associated. This disequilibrium between the four genes was only found when the total population was analyzed. Results were variable for subpopulations in which the number of animals was lower.

Analysis of the populations' structure and genetic differentiation

Analysis of molecular variance. The analysis of molecular variance (AMOVA) included only 9 of the 11 populations. The populations located in Envigado and Marinilla were excluded because of their low number of genotyped individuals. The AMOVA (Table 2) indicates that variation among populations was 1.5%, whereas variation within them was much higher (98.4%).

Table 2. Analysis of molecular variance (AMOVA) for Holstein cow populations in Antioquia (Colombia).

Source of variation	Sum of squares	Variance components	Percentage of variation
Between populations	826.3	0.01399	1.50976
Within populations	789.5	0.91280	98.49024
Total	1615.8	0.92679	

The calculated population-structure parameters were FIS, FST, and FIT. The FIS statistic was estimated specifically for each subpopulation. Results show a clear trend towards a negative value ranging between -0.222 and -0.627. This is indicative of an excess of heterozygotes, which could be related to a low level of inbreeding. Despite this negative trend, no case was found whose FIS value was significantly different from zero. The estimated absolute FIS values for the entire population were -0.022, 0.072, -0.021, and 0.006 for PRL, KC, BGH, and BoLA genes, respectively. This indicates a trend towards equilibrium because homozygotes and heterozigotes were in the expected frequencies. However, its value was not significantly different from zero for any of the cases ($p>0.05$). According to this result, the populations were generated by outbreeding (Hartl and Clark, 1989).

The FIT statistics value for the total population, including all the loci, was -0.0235. This, however, was not significantly different from zero ($p>0.05$). When the analysis was done independently for each loci the FIT values were: -0.020, 0.091, -0.020, and 0.000 for PRL, KC, BGH, and BoLA genes, respectively. Only the KC value was significantly different from zero ($p<0.05$), thus indicating a slight trend towards homozygosity in this gene.

The FST statistics yielded 0.045 when estimated for the entire population and all the loci were included. The same parameter had a p-value = 0.010, 0.107, 0.004, and 0.035 for PRL, KC, BGH, and BoLA genes, respectively. In all cases, except for KC, this value was significantly different from zero ($p<0.05$) indicating that a population structure does exist and the populations represented by those municipalities are genetically distinct, albeit with a low differentiation.

Nei distances

The estimated Nei distances between different populations indicated that those with the greatest genetic distance were located in Marinilla, Rionegro, and Envigado. Although distances between populations were not very high, a genetic differentiation between Eastern and Northern municipalities was observed (Table 3). It was also determined that subpopulations with less genetic distance were geographically closer and related in terms of genetic management.

Table 3. Nei genetic distances between eleven subpopulations of Holstein cows in Antioquia, Colombia.

	Medellín	Belmira	Bello	Don Matías	Entrerríos	Envigado	La Unión	Marinilla	Rionegro	San Pedro	Santa Rosa
Medellín	0.000										
Belmira	0.017	0.000									
Bello	0.004	0.015	0.000								
Don Matías	0.016	0.008	0.017	0.000							
Entrerríos	0.026	0.009	0.023	0.025	0.000						
Envigado	0.084	0.117	0.098	0.111	0.108	0.000					
La Unión	0.010	0.015	0.007	0.013	0.037	0.113	0.000				
Marinilla	0.323	0.274	0.353	0.295	0.259	0.221	0.390	0.000			
Rionegro	0.117	0.060	0.122	0.091	0.049	0.210	0.138	0.136	0.000		
San Pedro	0.008	0.012	0.003	0.015	0.027	0.108	0.001	0.373	0.125	0.000	
Santa Rosa	0.060	0.033	0.047	0.044	0.016	0.150	0.069	0.295	0.069	0.057	0.000

A dendrogram created with the UPGMA method (Figure 1) indicates an association between all municipalities. Marinilla and Envigado were the farthest apart in the genetic tree. Medellín and Bello, as well as Entrerríos and Santa Rosa, were very close. This is perhaps a consequence of their geographic proximity and the objectives set by the dairy trading in each municipality and region. In the case of San Pedro and La Unión, the genetic closeness could be explained by the fact that they are two of the most technologically advanced municipalities in the dairy sector and, therefore, their genetic management conditions are quite similar (Figure 1).

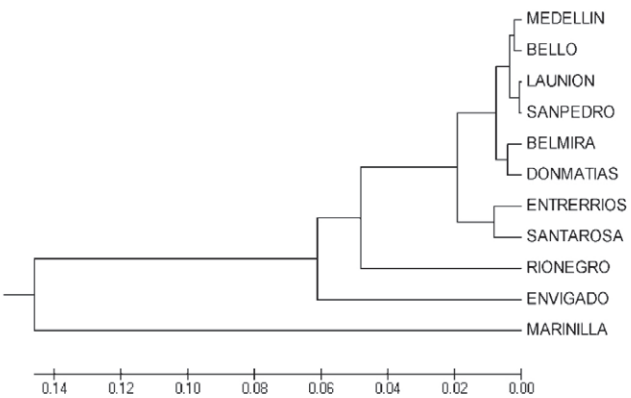

Figure 1. Dendrogram based on Nei's genetic distance for eleven subpopulations of Holstein cows in Antioquia, Colombia.

Discussion

Allele and genotype frequencies for BGH, PRL, and K-casein genes found in this study are consistent with Holstein studies in other regions of the world: 0.9 and 0.1 for BGH +/+ and BGH +/-, respectively (Brym, 2005); 0.31, 0.629, and 0.056 for PRLAA, PRLAB and PRLBB, respectively (Bukhari, 2013); 0.4, 0.34, and 0.26 for KCAA, KCAB, and KCBB, respectively (Hamza, 2010); and in Colombia (Solarte *et al.,* 2009; Zambrano *et al.,* 2010Echeverri *et al.,* 2010a; Echeverri *et al.,* 2010b). For BoLA DRB 3.2 gene, our study found fewer allelic and genotypic variants than Van Eijk *et al*. (1992) or Sharif *et al.* (2003). Colombian cattle genetics is the product of imported semen, mainly from the U.S. and Canada. This is why it is common to find similar allele and genotype frequencies among populations from Colombia and other countries.

The Hardy Weinberg equilibrium results for PRL and BGH genes are consistent with previous research in which there were no deviations from Hardy Weinberg equilibrium when working with a small population of Colombian Holstein cows genotyped for BGH and PRL (Echeverri *et al.,* 2010; Echeverri *et al.,* 2010b).

A study conducted in Colombia for some genetic groups found some degree of genetic differentiation

between the various breeds (Calvo *et al.,* 2009). Nevertheless, the Holstein breed was not included in that study.

Additionally, this study found that genetic similitude between Holstein cow populations in Antioquia is also due to their geographic proximity (60 km). San Pedro and La Unión are special case populations. These are the two most developed municipalities in terms of dairy production technology; therefore, their genetic management conditions are very similar.

The present study leads to the conclusion that Holstein cattle in Antioquia present genetic equilibrium despite having been subjected to indirect selection programs and inadequate methodologies. The effect of these programs has heavily impacted the gene frequencies for the studied markers. This could indicate that current improvements in phenotypic parameters result from management work and environmental changes. Breeding programs results are more notorious for herds rather than for the total population. It is necessary to implement a serious and reliable genetic breeding program to achieve better results and a stronger impact on phenotypic parameters.

Conflicts of interest

The authors declare they have no conflicts of interest with regard to the work presented in this report.

References

Addeo F, Chianese L, Di Luccia A, Petrilli P, Mauriello R, Anelli G. Identification of bovine casein variants by gel isoelectric focusing. Milchwissenschaft 1983; 38(10):586-588.

Aijun L, Xiucai H, Hong L, Chen A, Jihong J, Chunlei Z, Haixia X, Xueyuan G. Single nucleotide polymorphisms in bovine PRL gene and their associations with milk production traits in Chinese Holsteins. Mol Biol Rep 2010; 37:547-551.

Baranyi M, Bõsze S, Buchberger J, Krause I. Genetic polymorphism of milk proteins in Hungarian cattle breeds and PCR amplification of beta-lactoglobulin exon5 to identify genetic variant J by RFLP. 1997; [access date: August 17, 2014] URL:httphttp://agris.fao.org/agris-search/search.do?recordID=BE1999000171

Barroso A, Dunner S, Cânon J. Technical note: Use of PCR-single-strand conformation polymorphism analysis for detection of bovine β-casein variants A1, A2, A3, and B. J Anim Sci 1999; 77:2629-2632.

Brym P, Kamiñski S, Wójcik E. Nucleotide sequence polymorphism within exon 4 of the bovine prolactin gene and its associations with milk performance traits. J Appl Genet 2005; 46(2):179-185.

Bukhari S, Khan NN, Gupta P, Das AK, Raher GA, Chakraborty D, Pandey A. Prolactin gene polymorphism and its associations with milk production traits in Frieswal cow. Int J Mol Zool 2013; 3(3):10-13.

Calvo SJ, Martínez E, Tirado J, Corrales J, Montoya AE, Burgos WO, Cerón MF, Moreno M. Caracterización genética de las razas criollas BON y Romosinuano. LRRD 2009; [access date: October 10, 2011] URL: http://www.lrrd.org/lrrd21/4/calv21054.htm

Cabrera V, Rodríguez J, Méndez I, Larrea F, Chapé A. Caracterización bioquímica de prolactina de diferentes especies. Rev Cubana Endocrinol 2000; 11(2):78-89.

Chessa S. Development of a single nucleotide polymorphism genotyping microarray platform for the identification of bovine milk protein genetic polymorphisms. J Dairy Sci 2007; 90(1):451-464.

Chrenek P, Vasicek D, Bauerova M, Bulla J. Simultaneous analysis of bovine growth hormone and prolactin alleles by multiplex PCR and RFLP. Czech J Anim Sci 1998; 43:53-55.

Chikuni K, Nagatsuma T, Tabata T, Monma M, Saito M, Ozawa S, Ozutsumi K. Genetic variants of the growth hormone gene in Japanese cattle. Anim Sci Tech 1994; 65:340-346.

Dietz B, Cohen D, Timms L, Kehrli E. Bovine lymphocyte antigen class II alleles as risk factors for high somatic cell counts in milk of lactating dairy cows. J Dairy Sci 1997; 80:406-412.

Dietz B, Detilleux J, Freeman A, Kelley D, Stabe J. Genetic association of bovine lymphocyte antigen DRB3 alleles with immunological traits of Holstein cattle. J Dairy Sci 1997; 80:400-405.

Dybus A. Associations of growth (GH) and prolactin (PRL) genes polymorphisms with milk production traits in Polish Black and White cattle. Anim Sci Pap Rep 2002; 20(4):203-212.

Echeverri J, Vásquez N, Gallo Y. Efecto de la transición adenina/guanina del gen de la prolactina bovina sobre características de importancia en producción lechera. Rev Lasallista Investig 2010a; 7(2):16-23.

Echeverri J, Vásquez N, Gallo Y. Polimorfismo del gen de la somatotropina bovina y su asociación con características de importancia en la producción lechera. Rev Lasallista Investig 2010b; 7(1):58-65.

Eigel W, Butler J, Ernstrom C. Nomenclature of proteins of cow's milk. J Dairy Sci 1984; 67(8):1599-1631.

Ellegren H, Davies C, Andersson L. Strong association between polymorphisms in an intronic microsatellite and in the coding sequence of the BoLA-DRB3 gene – implications for microsatellite stability and PCR-based DRB3 typing. Anim Genet 1993; 24:269-275.

Falaki M, Gengler N, Sneyers N, Prandi A, Mássart S, Formigoni A, Burny A, Portetelle D, Renaville R. Relationship of polimorphisms for growth hormone and growth hormone receptor genes with milk production traits for Italian Holstein friesian bulls. J Dairy Sci 1996; 79:1446-1453.

Freeman M, Kanyicsca B, Lerant A, Nagy G. Prolactin: Structure, Function, and Regulation of secretion. Physiol Rev 2000; 80(4):1523-1631.

Ge W, Davis M, Hines H. Association of single nucleotide polymorphisms in the growth hormone and growth hormone receptor genes with blood serum insulin like growth factor I concentration and growth traits in angus cattle. J Anim Sci 2003; 81:641-648.

Gillespie B, Jayarao B, Dowlen H. Analysis and frequency of bovine lymphocyte antigen DRB3.2 alleles in Jersey cow. J Dairy Sci 1999; 82:2049-2053.

Gorodetskij S, Kaledin A. Analysis of nucleotide sequence of bovine K-casein. Genetic USSR 1987; 23:596-604.

Guo S, Thompson E. Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics 1992; 48:361-372.

Haldane JB. An exact test for randomness of mating. J Genet 1954; 52:631.

Hamza AE, Wang XL, Yang ZP. Kappa casein gene polymorphism in Holstein Chinese cattle. Pak Vet J 2010; 30(4):203-206.

Hartl D. A primer of populations Genetics. 3 ed. Sunderland (Mássachussets): Sinauer Associates Inc.; 2000. p. 26-31.

Ju Z, Li Q, Wang H, Li J, An O, Yang W, Zhong J, Wang F. Genetic polymorphism of κ-casein gene exon4 and its correlation with milk production traits in Chinese Holsteins. Yi Chuan 2008; 30(10):1312-1318.

Kusamura KM, Nassif SL, Martinez ML, Ferreira FP. Association of bGH and Pit-1 gene variants with milk production traits in dairy Gyr bulls. Pesq Agropec Bras 2004; 39(2):147-150.

Knight H. Overeview of prolactin'srole in farm animal lactation. Livest Prod Sci 2001; 70:87-93.

Lewin H, Schmitt K, Hubert R, Van Eijk M, Arnheim N. Close linkage between bovine prolactin and BoLA-DRB3 genes: mapping in cattle by single sperm typing. Genomics 1992; 13:44-48.

López E, Vásquez N. Determinación del sexo y genotipificación del gen de la kapa caseina en embriones bovinos. Rev Colomb Cienc Pecu 2004; 17(3):231-240.

Luccia DA, Addeo F, Corradini C, Mariani P. Bovine k Casein C: an electrophoretic study. Scienza e tecnica lattiero-casearia 1988; 39(6):413-422.

Lucy M, Hauser S, Eppard P, Krivi G, Clark J, Bauman D, Collier R. Variants of somatotropin in cattle: gene frecuencies in major dairy breeds and associated milk production. Domest Anim Endocrin 1993; 10:325-333.

Mackinlay A,Wake R. The heterogeneity of κ-casein. Biochim Biophys Acta 1964; 93:378-386.

Medrano J, Aguilar E. Genotyping of bovine kappa-casein loci following DNA secuence amplification. Biotechnology 1990; 8:144-146.

Mercier J, Brignon G, Ribadeau B. Structure primaire de caseine bovine. Sécuence Complète. Eur J Biochem 1973; 35:222-235.

Peakall R, Smouse P. GENALEX 6: Genetic Analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 2006; 6:288-295.

Penketh R, Delhanty A, Van Den Berghe J, Finklestone E, Handyside A, Malcolm S, Winston E. Rapid sexing of human embryos by non-radioactive in situ hybridization: potential for preimplantation diagnosis of X-linked disorders. Prenatal Diag 1999; 9:489-500.

Raymond M, Rousset F. Genepop version 1.2. population genetics software for exact tests and ecumenicism. J Hered 1995; 86:248-249.

Sambrook J, Fritsch E, Maniatis T. Molecular Cloning A laboratory manual. 2nd ed. Cold Spring Harbor Laboratory Press; 1989.

Sasavage N, Nilson J, Horowitz S, Rottman F. Nucleotide sequence of bovine prolactin messenger RNA. J Biol Chem 1982; 257:678-681.

Sharif S, Mallard B, Wilkie B. Characterization of naturally processed and presented peptides associated with bovine major histocompatibility complex (BoLA) class II DR molecules. Anim Genet 2003; 34:16-123.

Schneider S, Roessli A, Excoffier L. A software for population genetics data analysis. Genetics and Biometry Laboratory, Dept. of Anthropology and Ecology, University of Genova, Switzerland; 2000.

Sigurdardóttir S, Borsch C, Gustafsson K, Andersson L. Cloning and sequence-analysis of 14 DRB alleles of the bovine major histocompatibility complex by using the polymerase chain reaction. Anim Genet 1991; 22:199-209.

Solarte CE, Rosero CY, Eraso JM, zambrano GL, Cardenas H, Burgos WO. Frecuencias alélicas del gen Kappa caseína en la raza Holstein del trópico alto de Nariño – Colombia. LRRD 2009; [Access date: November 11, 2011] URL: http://www.lrrd.org/lrrd21/1/sola21003.htm

Soria A, Iglesias G, Huguet M, Mirande S. A PCR-RFLP Test to detect allelic variants of the bovine kappa-casein gene. Anim Biotechnol 2003; 14:1-5.

Takeshima S, Nakai Y, Ohta M. Short communication: Characterization of DRB3 alleles in the MHC of Japanese shorthorn cattle by polymerase chain reaction-sequence based typing. J Dairy Sci 2002; 85:1630.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, and Kumar S MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Mol Biol Evol. 2011; 28: 2731-2739.

Udina I, Karamysheva E, Sulimova G, Pavlenko S. Comparative analysis of Ayrshire and Black Pied cattle breeds by histocompatibility markers. Genetika 1998; 34:1668-674.

Van EM, Stewart H, Lewin H. Extensive polymorphism of the BOLA DRB3.2 gene distinguished by PCR-RFLP. Anim Genet 1992; 23:483-496.

Wicks J, Brooks C. Biological activity of phosphorylated and dephosphorylated bovine prolactin. Mol Cell Endocrinol 1995; 112:223-229.

Wojdak K, Kemic M, Strzalaka J. Prolactin gene polymorphism and somatic cell count in dairy cattle. J Anim Vet Adv 2008; 7(1):35-40.

Yao J, Aggrey D, Zadworny J, Hayes B. Sequence variations in the bovine growth hormone gene characterized by single strand conformation polymorphism SSCP analysis and their association with milk production traits in Holstein. Genetics 1996; 144:1809-1816.

Zakizadeh S, Rahimi G, Mirae S, Nejati A, Moradi M, Reinecke P, Reissmann M, Masoudi A, Amirinia S. Analysis of Bovine Growth Hormone Gene Polymorphisms in Three Iranian Native Breeds and Holstein Cattle by RFLP-PCR. Biotechnology 2006; 5: 385-390.

Zambrano GL, Eraso JM, Solarte CE, Rosero CY. Kappa casein genotypes and curd yield in Holstein cows. Relación entre los genotipos de la kappa caseína de vacas holstein y el rendimiento en cuajada. Rev Colomb Cienc Pecu 2010; 23(4):422-428.

Zhang H, Brown D, Denise D, Ax R. Nucleotide sequence determination of a bovine somatotropin allele. Anim genet 1993; 23:578.