GENETIC DIVERSITY AND PHYLOGENETIC RELATIONSHIPS BASED ON MILK PROTEIN GENETIC VARIANTS IN ROMANIAN SPOTTED, HOLSTEIN FRIESIAN AND MONTBÉLIARDE COWS

A.C. Grădinaru[1*], Daniela Elena Ilie[2], Şt. Creangă[1,3]

[1]*University of Agricultural Sciences and Veterinary Medicine of Iaşi, Romania*
[2]*Reasearch and Development Station for Bovine Arad, Romania*
[3]*Reasearch and Development Station for Bovine Iaşi, Romania*

Abstract

The present study aimed to analyze the milk protein genetic diversity in three of the most common breeds in Romanian farms (Romanian Spotted, Holstein Friesian and Montbéliarde). It was also tried to find possible relationships among the studied breeds solely using the milk protein polymorphism information. Therefore, the genetic similarity index using the Mayala-Lingsterm test and the magnitude of genetic divergence between two populations as Nei's genetic distance were calculated. In order to establish the genetic diversity, the homozygotic coefficient based on the homozygotic genotypes frequencies was statistically interpreted. The results showed that the genetic distance between the Romanian Spotted and Holstein Friesian breeds was lower than the genetic distance between the Romanian Spotted and Montbéliarde ones. The genetic distance between the Holstein Friesian and Montbéliarde breeds showed a slightly higher value than that between the Romanian Spotted and Montbéliarde ones. The homozygotic coefficient ranged from 22.50 to 28.47%. These results are normally considered for every panmictic population which is in Hardy-Weinberg equilibrium.

Key words: cattle breeds, genetic diversity, phylogenetic relationships

INTRODUCTION

Considering the milk protein behavior under the influence of the electrophoresis electric field, various genetic variants in different cattle breeds were identified up to now (8,15). Allelic variants of milk protein genes have a significant impact to the genetic improvement of dairy cattle breeds, most of them being associated with the yield traits, milk composition and milk properties. At the same time, the milk protein polymorphism may be used to establish the identity of each animal, the degree of relatedness between populations, as well as the participation rate of different breeds in the crossing process. Therefore, using the milk protein genes as a marker in the process of animal selection, and the integration of certain alleles in a National

Selection Program for Bulls, would have an economically positive impact both on the dairy product market and the conservation of the genetic background of the valuable individuals. Not in the least, the possibility of using the milk protein genetic variants in order to certify the dairy products origin, especially that of the traditional ones, should be encouraged increasingly more (4,5).

Many investigations on the certain milk protein genetic variants and yield traits have been done in Romania (1,2,10,12,13,14). Some correlations with the mastitis incidence in Holstein Friesian and Montbéliarde cows were established too (11). As a pioneering research work in Romania, the frequencies of about ten haplotypes for each of Romanian Spotted, Holstein Friesian and Montbéliarde population were identified; considering the IEF (isoelectric focusing) technique, allelic variants at the $\alpha_{S1}-$, $\beta-$, and $\kappa-CN$ (caseins) loci were determined (10). The genetic diversity among Romanian cattle breeds with

a special focus on the Romanian Grey Step Breed was studied by Georgescu et al. 2009 based on allelic frequencies for eleven different microsatellite loci.

The present study aimed to analyze the milk protein genetic diversity in three of the most common breeds in Romanian farms (Romanian Spotted, Holstein Friesian and Montbéliarde) and to establish possible relationships among the studied breeds using but information about milk protein polymorphism.

MATERIAL AND METHODS

This work is an extended statistical interpretation of the 435 IEF results of milk samples collected from 184 Romanian Spotted cows, 189 Holstein Friesian and 62 Montbéliarde cows. The analyses were performed at the Genetic Laboratory of Animal Breeding and Genetics Department, Justus Liebig University, Gießen, Germany according to the method reported by Erhardt, 1989, 1993 and previously published (10).

The *genetic similarity index (r)* between two populations using the Mayala-Lingsterm test was calculated (3). *„r"* was derived with the following formula:

$$r = \sum x_1 * x_2 / \sqrt{\sum x_1^2 * \sum x_2^2},$$

where, x_1 and x_2 refer to the allele frequencies for the αS1-CN, β-CN, κ-CN and β-LG (lactoglobulin) in the investigated

cattle populations. The *„r"* values can range from null to one; in the case that the studied populations have no phylogenetic relationship the *„r"* values will tend to null. As a confirmation for these results, the magnitude of genetic divergence between two populations, the Nei's genetic distance (16), was calculated using the PopGene version 1.31 (17).

In order to establish the genetic diversity, *the homozygotic coefficient (SH)* based on the homozygotic genotypes frequencies was calculated. *„SH"* was derived with the Helderman formula:

$$SH = \sqrt{\sum (H_i - \overline{H})^2 / n},$$

where, H_i refer to the level of homozygosis for each *locus*, $\overline{H}$ refer to the homozygosis average for the studied *loci* and *n* refer to the number of studied loci. The homozygotic coefficient could range from null to one hundred percent, considering the identical alleles ratio in the studied cattle population.

RESULTS AND DISCUSSIONS

The *genetic similarity index* results, according to the Mayala-Lingsterm's method, and the genetic distances, according to the Nei's method, for each two cattle populations taken in survey, are shown in table 1.

Table 1 Phylogenetic relationships based on milk protein genetic variants in Romanian Spotted, Holstein Friesian and Montbéliarde cows

Breeds	Mayala-Lingsterm similarity index			Nei's genetic distance		
	Romanian Spotted	Holstein Friesian	Montbéliarde	Romanian Spotted	Holstein Friesian	Montbéliarde
Romanian Spotted	****	-	-	****	-	-
Holstein Friesian	0.9950	****	-	0.0046	****	-
Montbéliarde	0.9574	0.9550	****	0.0426	0.0453	****

The results obtained for the Mayala-Lingsterm index showed that all three studied populations are very similar regarding the milk protein variants (r > 0.9). Nevertheless, some observed variations of this index could allow the following assessments: the highest values of the genetic similarity index could be observed between Romanian Spotted and Holstein Friesian populations while the

lowest values could be observed between Holstein Friesian and Montbéliarde populations. These results was confirmed by those for the genetic distance between the investigated populations. Thus, the genetic distance between the Romanian Spotted and Holstein Friesian subjects was lower than the genetic distance between the Romanian Spotted and Montbéliarde breeds. The

genetic distance between the Holstein Friesian and Montbéliarde breeds showed a slightly higher value than that between the Romanian Spotted and Montbéliarde ones.

The results for the genetic distance measurement, didn't confirm our expectations. The Romanian Spotted and Montbéliarde breeds belong to the Simmental family and the Holstein Friesian has a different phylogeny from these two cattle breeds. Nevertheless, the small number of the individuals and the few genetic markers investigated could influence the obtained results. However, a possible explanation for the higher similarity of the allelic variants identified for caseins and whey milk proteins between Romanian Spotted and Holstein Friesian populations is the fact that since the middle of the 19th century more Red

Holstein bulls were used for crossbreeding with Simmental cows as happened in 1970's in Montbéliarde breed. The conflicting results based on the phylogenetic relationships between the investigated populations demonstrate the necessity of a National Bulls Register to prove origins for each bull included into a reproduction program and the participation quota of every breed in the constitution of every bull. Only in this way, the genetic quality improvement of animals will be based on solid knowledge and gene pool preservation.

The homozygous genotypes quota and the homozygotic coefficient based on the homozygotic genotypes frequencies at the polymorphic *loci* in the investigated cattle populations are shown in table 2.

Table 2 The genetic diversity based on milk protein genetic variants in Romanian Spotted, Holstein Friesian and Montbéliarde cows

Breeds	Locus	The homozygous genotypes quota (H_i)%	The homozygotic coefficient (SH)%
Romanian Spotted	αS1-CN	89.67	22.50
	β-CN	51.09	
	κ-CN	61.96	
	β-LG	46.20	
Holstein Friesian	αS1-CN	98.94	23.02
	β-CN	50.79	
	κ-CN	57.67	
	β-LG	52.91	
Montbéliarde	αS1-CN	93.55	28.47
	β-CN	35.48	
	κ-CN	38.71	
	β-LG	51.61	

Heterozygosity is one of the most important genetic diversity parameter for a certain population. It is in a perfect relationship with homozygosity; the increasing of heterozygosity induces the decreasing of homozygosity and vice-versa. Investigations of the three catle populations shown that the homozygotic coefficient ranged from 22.50 to 28.47%. In fact, a homozygosity increasing close to 100% is due to the homozygous genotypes increase in the studied population, most likely as a result of inbreeding. Inbreeding leads to deviations from Hardy-Weinberg equilibrium by causing a deficit of heterozygotes. The investigated populations were already tested for Hardy-Weinberg equilibrium and the results shown that they were in equilibrium. Therefore, the

results obtained for the homozygotic coefficient are normally considered for every panmictic population.

CONCLUSIONS

The statistical interpretation of the IEF analysis results obtained of the milk samples collected from Romanian Spotted, Holstein Friesian and Montbéliarde cows showed that all three studied populations were very similar for the milk protein variants (r > 0.9). Nevertheless, the highest values of the genetic similarity index were observed between Romanian Spotted and Holstein Friesian populations while the lowest values were between Holstein Friesian and

Montbéliarde populations. This results were confirmed using the Nei's method. It was assumed that an explanation for these facts could be the use of the Red Holstein bulls for crossbreeding both with Simmental and Montbéliarde cows in the past.

The homozygotic coefficient results demonstrated a proper selection of the bulls used in the breeding program, avoiding the inbreeding as much as possible.

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