Scientific Papers, Animal Science, Series D, vol. LV
CD-ROM ISSN 2285-5769, ISSN-L 2285-5750

MARKERS IN A BLACK SPOTTED CATTLE POPULATION

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Abstract

The study of the genetic markers and identifying new markers involves an increasing number of research projects in the fields of genetics of immunology, biochemical genetics, molecular genetics, quantity genetics and the genetic improvement of animals.Having considered the importance of genetic polymorphism of biochemical structures we considered a study of the genetic characterization of a sample in a cattle population, based on the information offered by the genetic polymorphism at hemoglobin and transferrine loci and the analysis of the serum. Two phenotypic category, hemoglobin A (79,4%) and AB (20,6%), has been identified for hemoglobin locus. Five categories of individuals, homozygous for Tf^A/Tf^A (2.9%), Tf^D/Tf^D (58,8%) and heterozygous for Tf^A/Tf^D (20.6%), Tf^A/Tf^E (5,9%), Tf^D/Tf^E (11,8%) have been identified on seric transferine loci; determined of existence of interest locus for three kinds of genes Tf^A, Tf^D and Tf^E with codominant effect. Blood factors were described in order to use them as genetic markers with the purpose of determine the homogenity or heterogenity degree for two cattle population.Serologic relations were observed between the factors of B system. The most relevant was the complex BGK. These tree factors were observed in five different combinations, namely BGK, BG, B, G, and their total absence.

Key words**:** *reagents, bloods phenogroups, immuno-serological, haemoglobin, transferinne*

INTRODUCTION

Theoretical and practical achievements in husbandry are continuously conditioned in a great extent by the genetics evolution as a biology field.

Economical and food crisis as a result of the contemporary demographic explosion, leads to the fact that the material resources obtaining and revaluation will become the major objective of the „biological revolution", which will affect humanity much deeper than „mechanical revolution" of the XIX –th century or technological revolution of our century.

The complex knowledge and using of the high productive potential of living matter need fundamental and appliable researches, finally justified by a high efficiency obtained with minimum expenses.

In this context, genetics offer to the mankind new possibilities, both by reevaluating natural resources by animal genetical improvement , and by creating new organisms using modern biotechnologies [2,6].

One of these possibilities is offered by genetic markers, which are object of several researches within immunogenetics, biochemical genetics, molecular genetics and even quantitative genetics fields [1,3].

MATERIAL AND METHOD

The studied material included 34 individuals of Black spotted cattle population.

The blood samples collection was accomplished in heparinized, standard test tubes. Determination of blood phenogroups was realized according to the standard methodology, by using the set of 44 reagents existing in the immuno-serology laboratory. To realize the hemolytic test, Plexiglas plates were used, with buckets of 7 mm depth and 5 mm diameter, into which was dropped a drop from each monoserum used to test the eritrocitary suspensions. Then it is added, over these, a drop from the searched eritrocitary suspension; afterwards, the plates were shaken to realize the mixture between the antiserum and the erythrocytes in the suspension. After 10

minutes rest at room temperature, it was dropped over the mixture in each bucket a drop of complement. After the three components were introduced in the buckets, the plates were shaken again; afterwards they were incubated at 25^0C. The reaction reading was realized at ½ hours from incubation, at 2½ hours and at 5 hours. After each reading the plates were shaken [2].

The various degrees of hemolysis realizing were estimated by reading:

-negative reaction: all erythrocytes are deposited, the above liquid is clear;

-positive reaction: it was appreciated according to the lysed red cell utilized in the following four values scale: light hemolysis, accentuated hemolysis, net hemolysis, complete hemolysis.

For establishing the types of hemoglobin, we used the technique of vertical electro-phoresys, using polyacryl amidae as a migration support, the same technique as used by Meriaux J.C. (1992), adapted to the conditions in the bio-chemistry laboratory the Faculty of Biology of The University of Bucharest [8].

RESULTS AND DISCUSSIONS

The locus of haemoglobin

Statistical analysis of the studied sample revealed two types of haemoglobin, namely A and AB genetically determined by the following genotypes: homozygous Hb^A/Hb^A and heterozygous Hb^A/Hb^B. Identification of genotypic categories on haemoglobin locus allowed establishing genetic structure within this sample. Homozygous Hb^A/Hb^A individuals presents the highest occurrence within genetic structure, namely four times higher than heterozygous ones (Fig. 1).

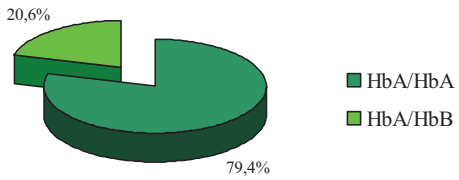

Fig. 1. The genotypic category distribution on haemoglobin locus (%)

The presence of two genotype categories in the group demonstrates the presence of two categories of genes, Hb^A, Hb^B, identified with different frequency (Fig. 2).

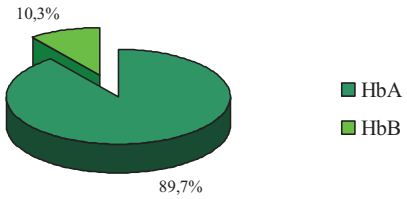

Fig. 2. Gene category distribution haemoglobin locus (%)

Hb^A gene was determined with a high frequency (89,7%), respectively compared to about 9 times higher than Hb^B.

The analysis of this sample equilibrium state, using χ^2 test (table 1), lead to the conclusion that this sample has a balanced genetic structure. We may notice that because after comparing table value χ^2 (5,99) with calculated value χ^2, the calculated value is smaller comparative with table value.

Table 1. *Equilibrium state estimation on haemoglobin locus*

Genotypes	No. observed genotypes	No. expected genotypes	d^2/A
Hb^A/ Hb^A	27	27,36	0,005
Hb^A/ Hb^B	7	6,28	0,083
Hb^B/ Hb^B	0	0,72	0,360
Total	34	34	χ^2 =0,448

The locus of serum transferines

The interpretation of electrophoresis graphs for the 34 individuals has detected five categories of individuals: homozygous for genes Tf^D, Tf^E, heterozygous Tf^A/Tf^D, Tf^A/Tf^E and Tf^D/Tf^E (figure 3).

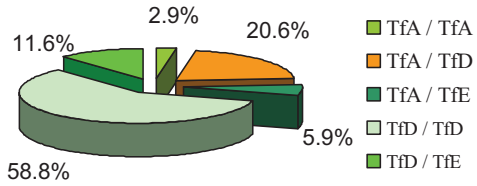

Fig. 3. The genotypic category distribution on transferrine locus (%)

The highest share was found in heterozygous individuals Tf^D/Tf^D (58,8%), with 55,9% higher than homozygous Tf^A/Tf^A (2,9%), which were emphasized with the smallest frequency.

The presence in the analyzed population of five genotypic categories emphasize that three gene categories, namely Tf^A, Tf^D and Tf^E, existed in the analyzed sample identified with different frequencies.

According to cathegory distribution, no homozigous Tf^E/Tf^E individual has been found in this sample. From this reason, after the gene category frequency determination, the gene Tf^E showed the lowest percent (figure 4)

Tf^D gene is characterized by a frequency about 77% higher than the frequency of gene Tf^E, which was described with the lowest procent.

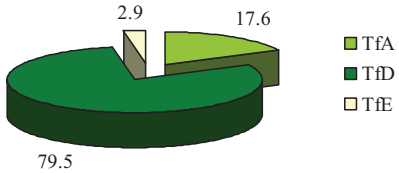

Fig. 4. Gene category distribution transferrine locus (%)

Determining the ratios of the genotype categories and, as a result, the genetic structure of the sample in this study allowed building an estimate for the status of genetic equilibrium, for locus analyzed.

The results of equilibrum state estimation for this sample show a balanced genetic structure, calculated χ^2 value (1,650) being smaller than the table value (5. 99). Results are presented in table 2.

Table 2. Equilibrum state estimation on transferrine locus

Genotypes	No. observed genotypes	No. expected genotypes	d^2/A
Tf^A/ Tf^A	1	0,887	0,014
Tf^A/ Tf^D	7	8,236	0,185
Tf^A/ Tf^E	2	0,972	1,087
Tf^D/ Tf^D	20	19,125	0,040
Tf^D/ Tf^E	4	4,514	0,058
Tf^E/ Tf^E	0	0,266	0,266
Total	34	34	χ^2 =1,650

In this effective there were not emphasized individuals with the I_2, E'_3 and G'' factors.

The M factor is associated with a low milk production. This factor was noticed at 2,9% of individuals [7,9].

For 13% from the emphasized erythrocytary, a net hemolytic was obtained. The most positive reactions (79%) were of complete hemolytic. For the rest of positive reactions (8%) it was found that the hemolytic was of 50%, the above liquid being pink-reddish colored.

The most reactions were observed within system B (table 3), known as the most complex blood group system at cattle [5]. The most relevant was the complex BGK. These tree factors were observed in five different combinations, namely BGK, BG, B, G, and their total absence. Another type of relations, namely the linear relations were observed between the factors of systems A (A_1, A_2), B (G_1, G_2, G_3; O_1, O_2, E'_1, E'_2), C (C_1, C_2; X_1, X_2), FV (F_1, F_2). The genetic explanation of these subtypes of blood group factors consists of the different antigenic structure of the respective factors. Between the subtypes of the same factor crossed reactions may appear, these being characteristic for each system.

Table 3. Share of blood group systems category

Blood group system	Factor of blood group system	N 34	Factors distribution of blood group system
A	A_1	31	0,911
	A_2	27	0,794
B	B	10	0,294
	G_1	15	0,411
	G_2	25	0,735
	G_3	21	0,617
	I_1	26	0,764
	K	9	0,264
	O_1	27	0,794
	O_2	19	0,558
	T_1	13	0,382
	Y_2	27	0,794
	D'	23	0,676
	E'_1	23	0,676
	E'_2	25	0,735
	O'	20	0,588
	G'	32	0,941
	I'	19	0,558
	J'	14	0,411
	Q'	26	0,764
	Y'	17	0,500
C	C_1	18	0,529
	C_2	10	0,294
	E	23	0,676
	R_1	8	0,235
	W	29	0,852
	X_1	10	0,294
	X_2	28	0,823
	L'	27	0,794
FV	F_1	25	0,735
	F_2	1	0,029
	V	16	0,470
J	J	9	0,264
L	L	21	0,617
M	M	1	0,029
SU	S	28	0,823
	H'	32	0,941
	H''	16	0,470
	U'_1	4	0,117
	U''	3	0,088
Z	Z	30	0,882

CONCLUSIONS

1. Two categories of individuals have been described within the lot and they are as following: homozygous Hb^A/Hb^A and heterozygous Hb^A/Hb^B. The homozygous individuals Hb^A/Hb^A represent more than two thirth of the sample
2. The polymorphism study from the seric protein locus pointed out five genotypic categories: Tf^A/Tf^A, Tf^A/Tf^D, Tf^A/Tf^E, Tf^D/Tf^D and Tf^D/Tf^E
3. Following the analysis of the genetic balance conditioner seric transferine and haemoglobin locus, was concluded that a genetic balance condition already exist for the analyzed population.
4. The most reactions were observed within system B, known as the most complex blood group system at cattle.
5. The lowest gene frequency was present in F_2 and M factors (2,9%), and highest one in G' and H' (94,1%).

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