

Genetic Investigations Using Immuno-biochemical Markers in a Maramureş Brown Cattle Population

**Nicoleta Isfan¹, Vasile Bacila¹, Dana Popa¹, Sergiu Georgescu², Razvan Popa¹,
Monica Marin¹, Carmen Nicolae¹, Marius Maftai¹**

¹ *University of Agricultural Sciences and Veterinary Medicine of Bucharest, Faculty of Animal Science, 011464, Bucharest, Bdul Mărăşti, nr. 59, sect. 1, Romania*

² *University of Bucharest, Faculty of Biology, 050095, Bdul. Splaiul Independentei, nr. 91-95, sector 5, Romania*

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. Some studies on genes frequency determining the red cells specificity and for why hemoglobin are approached in the present report. In this way, some blood factors, most of them belonging to B system (the most complex system in cattle) have been evidenced. The lowest gene frequency was present in K factor (7%), and highest one in, O₁, G⁻, W and F₁ (100%). In addition to basic importance on knowledge and determination of cattle population genetic structure for studied protein loci, another theme proposed to correlate hemoglobin type with some traits of economical importance: milk yield, fat and protein content, fat and protein yield. Higher performance was recorded by Hb^A/Hb^A individuals.

Keywords: genetic polymorphism, immuno-biochemical markers, genetic structure, traits.

1. Introduction

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.

Some studies on genes frequency determining the red cells specificity and for why hemoglobin are approached in the present report. In this way, some blood factors, most of them belonging to B system (the most complex system in cattle) have been evidenced [1].

In addition to basic importance on knowledge and determination of cattle population genetic structure for studied protein loci, another theme

proposed to correlate hemoglobin type with some traits of economical importance: milk yield, fat and protein content, fat and protein yield.

2. Materials and methods

The studied material included 36 individuals of Maramureş Brown 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 40 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

* Corresponding author: Nicoleta Isfan, Tel 004 0745 07 25 64, Fax 004021 318 28 88, Email ndefta@yahoo.com

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°C. 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 [3].

The ranging of individuals – according to the loci of the studied proteins and the four analyzed traits – was established consequently the statistic determinations.

3. Results and discussion

In this effective there were not emphasized individuals with the G₁, I₂, E₃, G'', X₁, F₂ and H'' factors.

The M factor is associated with a low milk production [4]. This factor was noticed at 6.7% of individuals (Table 1).

Table1. Distribution the system of blood groups

System	Factors of blood groups	Factors frequencies	Blood group frequencies
A	A ₁	0.60	0.054
	A ₂	0.43	
B	B	0.60	0.487
	G ₂	0.47	
	G ₃	0.37	
	I ₁	0.27	
	K	0.07	
	O ₁	1.00	
	O ₂	0.77	
	T ₁	0.77	
	Y ₂	0.93	
	D'	0.37	
	E' ₁	0.67	
	E' ₂	0.57	
	O'	0.47	
	G'	1.00	
	I'	0.63	
J'	0.43		
Q'	0.97		
Y'	0.87		
C	C ₁	0.63	0.189
	C ₂	0.60	
	E	0.60	
	R ₁	0.40	
	W	1.00	
	X ₂	0.80	
	L'	0.97	
FV	F ₁	1.00	0.054
	V	0.40	
J	J	0.533	0.027
L	L	0.200	0.027
M	M	0.067	0.027
S	S	0.433	0.108
	H'	0.970	
	U' ₁	0.300	
	U''	0.130	
Z	Z	0.430	0.027

For 11% from the emphasized erythrocytary, a net hemolysis was obtained. The most positive reactions (86%) were of complete hemolysis. For the rest of positive reactions (3%) it was found that the hemolysis was of 50%, the above liquid

being pink-redish colored. The most reactions were observed within system B, known as the most complex blood group system at cattle (figure 1).

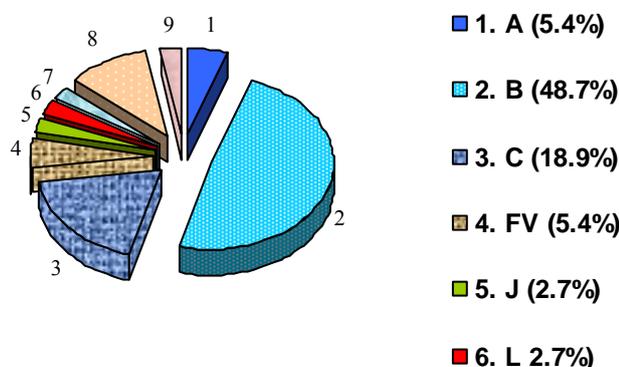


Figure 1. Share of blood group systems category

The locus of hemoglobin

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 [5].

The two genetic categories are genetically determined by the presence, at the serum pre-

albumins locus, of two categories of genes, Hb^A and Hb^B (table 2).

The homozygous individuals Hb^A/Hb^A represent more than one third of the sample (figure 2).

Table 2. Distribution of gene and genotype categories at hemoglobin locus

Genotype categories	N	The ratio of genotype categories (%)	The distribution of gene categories (%)	
			Hb^A	Hb^B
Hb^A/Hb^A	31	86.11	93.055	6.945
Hb^A/Hb^B	5	13.89		

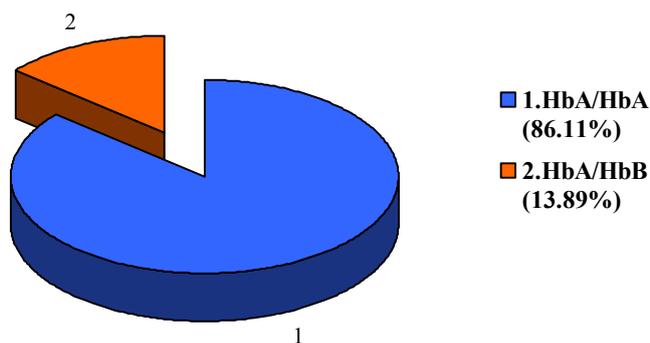


Figure 2. Share of genotypic categories on seric hemoglobin locus

Table 3. Average performances according to hemoglobine locus genotype

Genotype	N	Milk yield (l) $\bar{X} \pm S \bar{X}$	Fat percent $\bar{X} \pm S \bar{X}$	Fat yield $\bar{X} \pm S \bar{X}$	Protein percent $\bar{X} \pm S \bar{X}$	Protein yield $\bar{X} \pm S \bar{X}$
Hb^A/Hb^A	31	3951.30±99.73	4.05±0.04	160.21±4.18	3.44±0.015	136.08±3.23
Hb^A/Hb^B	5	3794.30±200.53	4.13±0.10	156.21±7.59	3.39±0.04	129.13±7.35

Average milk production traits have been established taking into account haemoglobin locus genotype: milk yield, fat and protein percentage, fat and protein yield. The obtained results are presented in Table 3.

Hemoglobin A individuals recorded the highest milk yield. Heterozygous individuals yielded 157 l lesser milk than homozygous ones. As regards fat percentage, a quite small difference (0.08% only), can be observed.

Genetically, milk yield is negatively correlated with fat percentage, in agreement with our expectations.

The highest fat yield has been recorded by hemoglobin A individuals, approximately 4g more than hemoglobin AB individuals [6].

As regards protein percentage, a quite small difference has been detected between the two categories.

Hb^A homozygous individuals recorded the highest average protein yield, 6.95 g more than heterozygous individuals.

A simultaneous study for the fifth analyzed traits reveals the highest performing capacity in hemoglobin A individuals which were placed in the top of the rank in four analyzed traits, namely milk yield, fat yield, protein yield and protein percentage.

Conclusions

1. The most reactions were observed within system B, known as the most complex blood group system at cattle.
2. The lowest gene frequency was present in K factor (7%), and highest one in, O₁, G₁, W and F₁ (100%).
3. 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 one third of the sample
4. Hemoglobin A individuals recorded the highest milk yield.
5. The highest fat yield has been recorded by hemoglobin A individuals
6. Hb^A homozygous individuals recorded the highest average protein yield.

Acknowledgements

"This work was cofinanced from the European Social Fund through Sectoral Operational Programme Human Resources Development 2007-2013, project number POSDRU/89/1.5/S/63258 "Postdoctoral school for zootechnical biodiversity and food biotechnology based on the eco-economy and the bio-economy required by eco-san-genesys"

References

1. Bell, K., The blood groups of domestic mammals. In Agar, N.S. and doard, P.G. (eds) Red Blood Cell of Domestic Mammals. Elsevier Science Publishers B.V. Amsterdam, 1983, pp. 133-164
2. Granciu, I., Cureu, I., Duică S., Grupele sanguine la animalele domestice, Ed. Ceres, București, 1973,
3. Mariaux, J. C., Control de filiation et marqueurs sanguines chey les equides. Rec.Med.Vet. Special Reprod.des Equides, 1992, pp.17-24
4. Larsen, B., Jensen, N. E., Association of the M blood groups system with bovine mastitis. Animal blood Groups and Biochemical Genetics, 1985, 16, pp. 165-173
5. Hines, H. C., Blood groups and biochemical polymorphisms. The genetics of cattle, 1999, pp. 77-108
6. Neimann-Sorensen, A., Robertson, A., The association between blood groups and several production characteristics in three Danish cattle breeds. Acta Agriculturae Scandinavica, 1961, 11, 163-196