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GENETIC DIVERSITY IN THE ROMANIAN SHEEP BREEDS QUANTIFIED BY MEANS OF INFORMATIONAL ENERGY

Gheorghe HRINCĂ

Research and Development Station for Sheep and Goat Breeding, Popauti,
Rachiti Village, Botosani County, 717310, Romania

Corresponding author email: ghrinca@yahoo.com

Abstract

*The study makes an analysis of the genetic diversity (**d**) in the Romanian sheep breeds (Palas Merino, Tsigai, Tsurcana and Botosani Karakul) using a concept of informational statistics termed the informational energy (**e**) which measures very precisely the genetic heritage of the taxonomic entities than by common indicators of mathematical statistics (allelic frequency analysis, significance testing etc.). The informational-statistical quantification of genetic diversity used the allelic frequencies within three genetic system types in the calculus algorithm: five biochemical-genetic systems (haemoglobin, transferrin, albumin, amylase, blood potassium), six immunogenetic systems (blood group systems A, B, C, D, M, R-O) and seven genetic-molecular systems (β-lactoglobulin, calpastatin, α_s1-casein, prion, SLS, Booroola, Inverdale). The accuracy of the new approach on the genetic diversity resides from a complex analysis of the allele number, their frequencies, inter allelic ratio and genetic polymorphism degree within each system. The paper describes the genetic diversity levels both within each system, as well as in associated systems, and highlights the genetic diversity differences among the Romanian sheep breeds in relation to the systemic parameters mentioned. Also, some comments are made about the heterozygosity degree of sheep breeds in corroboration with genetic diversity. Finally, the paper argues the importance of genetic diversity measured in terms of informational statistics for improvement and conservation programs in the field of farm animal breeding, but makes clarifications also about the danger that comes from increasing the diversity at some loci associated with genetic diseases.*

Key words: *Romanian sheep breeds, genetic marker, genetic diversity, informational energy*.

INTRODUCTION

Exploration, collection and scientific preservation of genetic resources from all types of agricultural holdings is an urgent action of the utmost importance in which all countries as well as numerous scientific and cultural forums and professional associations of livestock farmers must be involved through coherent state and non-governmental policies (Patterson, 2003). The technical coordinates of the World Programme of FAO, entitled Mo-DAD, has been focused on identification, description, development, use of animal genetic resources, conservation of unique or endangered resources, training, information and participation in the management of animal genetic resources and communication improving through dialogue and international connections to manage these resources. The project The Mo-DAD aims quantifying the genetic variation among the main domestic animal breeds based on molecular genetic evaluation and measuring the genetic distances among breeds, providing the first global information of relations among themselves or about the number and diversity of the breeds (Scher, 2012). Also, within the European Commission for Agriculture and Rural Development there is a committee issuing laws on the conservation, characterization, collection and utilization of genetic resources in agriculture (ECC, 2001; GRFAC, 2011; ECNB, 2011). Thus, it has been created an international scientific current of major attitude known as the management of genetic resources and biodiversity in animals (Galal and Hamond, 1996).

This challenge is achievable by introducing in the animal breeding practice of certain biological indicators expressing the animal inwardness for its genotypic individualization (biochemical and molecular markers) since certain of its external features or productivity traits (morphological markers) have a lower or sometimes even insignificant relevance. The expressions of certain biochemical-genetic (electrophoresis variants of proteins polymorphic or the flam photometric ones of some mineral

elements with discontinuous distribution in the blood), immunogenetic (antigens of blood group factors) and molecular-genetic (DNA sequences, microsatellites) structures, outside any influence of external or internal factors, have the quality of genetic markers that are highly relevant in describing the genetic individuality of an animal (Ordas and San Primitivo, 1986; Sargent et al., 1999; Moioli et al., 2006; Maddox and Cockett. 2007; McManus et al., 2010; Hrincă, 2016*a*).

All approaches regarding the genetic diversity assessment using the genetic markers took into account the comparative analysis of different alleles and genotypes within of some systems with multiple molecular forms, the observed and expected frequencies and testing the genetic equilibrium in animal populations. But, using these indicators of mathematical statistics is not a sufficient condition because they provide rough estimates on the genetic heritage of animal populations. These tools are provided by the informational statistics (Groza and Padeanu, 1999; Hrincă Groza, 2001; Hrincă, 2015, 2016). This is the reason of this paper to quantify as accurately as possible the genetic diversity of the Romanian sheep breeds by means of an informational-statistical concept, named *informational energy*, using various genetic markers in the calculus algorithm.

MATERIALS AND METHODS

The genetic diversity was analyzed within of some Romanian sheep populations belonging to four indigenous breeds: Palas Merino, Tsigai. Tsurcana and Botosani Karakul. To achieve this goal the allele frequencies from 18 polymorph systems were used: five biochemical-genetic systems (haemoglobin, transferrin, albumin, amylase and blood potassium), six immnunogenetic systems (blood group systems A, B, C, D, M and R-O) and seven molecular-genetic systems (β-lactoglobulin, calpastatin, α_s1-casein, prion, Spider Lamb Syndrome, Booroola and Inverdale). The polymorphism of genetic structures was highlighted by specific laboratory methods:

- biochemical-genetic systems - by starch gel electrophoresis methods (for polymorphic proteins) and by flam photometric method (for potassium ions with discontinuous distribution) (Hrincă, 2015);
- immunogenetic systems - by haemolytic assay method (for systems A, B, C, M and R-O) and by haemagglutination method (for system D) (Hrincă, 2015);
- molecular-genetic systems - by PCR-RFLP methods (Kevorkian, 2010; Lazar et al., 2015; Hrincă, 2015).

In terms of mathematical statistics the allelic frequencies were calculated within all genetic systems and the genetic equilibrium of animal populations has been estimated at the level of each locus by means of the χ^2 test.

Quantification of the genetic diversity (*d*) of each sheep breed was performed using the informational energy (*e*) in corroboration with the heterozygosity degree (*Ht*)) of their populations at the determinant loci of all polymorphic systems as a first indicator of diversity (Groza and Pădeanu, 1999; Hrincă and Groza, 2001; Hrincă, 2015).

The complex informational energy (e_t) and complex genetic diversity (d_t) were calculated for a broader perception of polymorphism level of all genetic systems (Hrincă and Groza, 2001; Hrincă, 2015).

RESULTS AND DISCUSSIONS

The calculus algorithm of genetic diversity (*d*) by means of informational energy (*e*) was based on the allele frequencies identified in the 18 genetic systems, their number being 70, as follows:

- 17 alleles within the *biochemical-genetic systems*: in the transferrin system nine alleles were found and the other four systems each contain two alleles (Table 1);
- 36 alleles within *immunogenetic systems*: two alleles for each locus of the 13 blood group factors (Table 2);
- 17 alleles within the *molecular-genetic systems*: the prionic system contains five alleles and the other systems are of biallelic type (Table 3).

The number of alleles, their dispersion and especially the distributional ratio among them within each system configure the value of the two indicators of informational statistics. The informational energy and genetic diversity quantified by informational statistics concepts are dimensionless sizes. Being complementary

sizes, both can take values from *0* to *1* depending on the share of all components within a certain system; the more the distributions show a wider variability, the more the informational energy increases and the genetic diversity decreases, and the more balanced these distributions are, the more the informational energy decreases and the genetic diversity increases (Groza and Padeanu, 1999; Hrincă and Groza, 2001; Hrincă, 2015).

All four Romanian sheep breeds are distinguished among them in terms of number of alleles, their distributions and of the polymorphism measure at the analyzed loci, structures that confers to each breed a distinct genetic profile which is reflected on its genetic diversity degree.

Diversity within the biochemical-genetic systems (Table 1)

In the haemoglobin system, the Hb^B allele is prevalent in all sheep breeds compared to its Hb^A codominant. Because of this, the informational energy coefficients record high values, their correspondence being reflected in low or moderate levels of diversity at the Hb locus. The lowest level of haemoglobin diversity occurs in Botosani Karakul (0.16). The Tsigai breed records a decreased haemoglobin diversity, too (0.2). In Tsurcana and especially in Palas Merino, in which the gap between the two Hb alleles are more diminished in comparison with the first two breeds, there is an increase in haemoglobin diversity, reaching 0.35 in Tsurcana and 0.40 in Palas Merino.

In the transferrin system, the richest genetic diversity is found in the Tsigai breed that contains all nine Tf alleles in their genotypic structures (0.98). The diversity is very large in Tsurcana and Palas Merino breeds, too. Although in Turcana there are seven alleles and Palas Merino breed has eight alleles, the transferrin diversity coefficient is slightly higher in Tsurcana (0.96) than in Palas Merino (0.92); this is because the Tf allele distributions of Tsurcana are more uniform than those of Palas Merino. In Botosani Karakul, the transferrin diversity, though it is appreciable, is lower (0.68) than in the previous breeds because there are only six alleles and especially because the share is held by alleles Tf^C (60%) and Tf^B (33%), the other alleles being sporadically encountered in this breed.

The albumin system of the Romanian sheep breeds is characterized by a very limited polymorphism because of the allele Alb^S fixing in an overwhelming proportion, especially by natural selection. Only the Palas Merino and Tsigai breeds present albumin polymorphism of binary type and Tsurcana and Botosani Karakul breeds are monotypic at the Alb locus. In Palas Merino and Tsigai the albumin panel is dominated by the Alb^S allele in a 95-96% proportion and only 4-5% is occupied by Alb^F allele. The albumin monotypism of Tsurcana and Botosani Karakul breeds consists in the existence of homozygous genotype for Alb^S allele only. For these reasons the informational energy coefficients are extremely high, which makes the albumin diversity to be very low, about 0.10 in Palas Merino and Tsurcana and completely absent in Tsurcana and Botosani Karakul (0).

Except the Tsgai breed, the other breeds are monotypic for Am^B allele in the amylase system. Therefore, the genetic diversity of this locus is zero in these three breeds. The amylase diversity is moderately manifested only in the Tsigai sheep (about 0.2) where the polymorphism is owed to the massive presence of Am^B allele (92%) and relatively low incidence of Am^C allele (8%).

Within the blood potassium system the genetic diversity is absent in the Palas Merino breed because the only present allele is the K^L dominant one. In the other three breeds there is kalium diversity because, in addition to K^L allele, its K^h recessive is also present. If in Tsigai sheep the K^L allele is predominant (about 94%), in Botosani Karakul breed the K^h allele has the highest value (about 92%). Although these two alleles occur with diametrically different frequencies in the two breeds, the genetic diversity at this locus is relatively low but almost similar in both breeds because the gap between the two alleles is similar, slightly more reduced in Botosani Karakul than in Tsigai; therefore the blood potassium diversity is slightly higher in Botosani Karakul (0.18) compared to that of Tsigai (0.15). In exchange, in Tsurcana due to very uniform spread of the two alleles, the genetic diversity at Ke locus is quite significant.

Diversity within the immunogenetic systems (Table 2)

The erythrocyte factors belonging to all six blood group systems are well represented having different frequencies for each sheep breed. In most cases, the frequencies of dominant alleles, that control the expression of blood factors, are lower than of their recessive variants that determine the absence of some factors. Can also find some cases when the dominant alleles had higher incidence than their recessive, this situation being met with predilection in Tsurcana sheep at the level of factors Aa, Bb, Bf, Ca, Cb, and Ma, Tsurcana having the richest immunogenetic dowry among all breeds. Also in Tsigai at Cb and Ma loci, in Botosani Karakul at Bb locus and in Palas Merino at Cb locus, the dominant alleles are most common than the recessive alleles. In one case there is an almost unitary ratio between the two allele types at the Bf factor level in Botosani Karakul. Due to the consistent frequencies of erythrocyte antigens within all six blood group systems, the informational energies have such sizes entailing the exteriorization of a considerable genetic diversity at each erythrocyte factor level in all sheep breeds, in most cases the scores ranging between 0.50 and 0.65 (tab. 2). The immunogenetic diversity measure is given by the distributions of one or other of the two allele types and especially by the ratios between their frequencies. As a general rule, for a good score of immunogenic diversity ranged between 0.55 and 0.65, the ratios between the allelic frequencies have to be 35% / 65% or 45% / 55%. If these ratios widen, then the immunogenetic diversity decreases: for example, at a ratio of 20% / 80% the diversity reaches approximately 0.4 and at a ratio of 10% / 90% it decreases to 0.10-0.15.

At the Aa factor level the immunogenetic diversity is significant in Palas Merino, Ţigaie (0.56) and Botosani Karakul (0.61) breeds. In exchange, the high informational energy generated by the large gap between the two alleles in Tsurcana leads to a low diversity.

In the system B, the most diverse locus is the one of factor Bb. In all breeds the diversity exceeds 0.5, especially in Palas Merino and Botosani Karakul. At the level of the other factors within B system the best diversity is recorded in Botosani Karakul, its value being between 0.55 and 0.62. Similar values of immunogenetic diversity are found in Tsurcana too, except for Bf factor (about 0.4). In the other two breeds, the diversity coefficient threshold at the factors of B system is below 0.5, some of them recording relatively low values between 0.2 and 0.25, such as in Palas Merino for Bd and Bg factors or in Tsigai for Bc factor. A more substantial diversity in the two breeds would be signalled at the Bf and even Bi factor level.

Within the system C, the Tsurcana and Botosani Karakul breeds possess a good diversity for Ca factor (around for 0.62); contrary, in Tsigai breed the diversity is moderate (0.19) or relatively low in Palas Merino (about 0.14). Unlike Ca factor, the Cb factor is more diverse in all breeds, the diversity value being more than 0.55, excluding the Tsurcana (0.45).

At the Da factor level, the highest diversity is found in Botosani Karakul and Tsurcana breeds (0.58-0.59). In the other two breeds the diversity is moderate, 0.41 in Tsigai and 0.31 in Palas Merino.

At the Ma factor level the immunogenetic diversity reaches significant levels in Tsurcana (0.55) and especially in Tsigai and Botosani Karakul (0.60-0.62), while in Palas Merino this coefficient is lower (0.4).

Within the R-O system, the O factor is more diverse than the R factor. At the R factor level, the diversity is slightly higher than the 0.5 threshold in Palas Merino and Botosani Karakul; in the other two breeds this coefficient is below 0.5, especially in Tsigai. On the contrary, for the O factor, the immunogenetic diversity value is at the 0.5 limit (in Palas Merino and Tsigai) or more over this limit (in Tsurcana and Botosani Karakul).

Diversity within the molecular-genetic systems (Table 3)

Within the β-lactoglobulin system, the LGB^A allele is more spread than the LGB^B allele in all breeds, but the gap between their distributions are not so large as to generate significant informational energies. Therefore, at the LGB locus we have a consistent diversity being most obvious in Palas Merino and Tsigai (0.62), slightly above the 0.5 threshold in Tsurcana and at the limit of this level in Botosani Karakul.

Table 1. Coefficients of informational energy (*e*), genetic diversity (*d*) and heterozygosity (*Ht*) at the loci of biochemical-genetic systems in the Romanian sheep breeds

Biochemical-genetic system	Breed	Allele	Allelic frequency	e	d	Ht
Haemoglobin system	Palas Merino χ^2= 0,6014	Hb^A	0.1930	0.6106	0.3894	0.3123
		Hb^B	0.8070			
	Tsigai χ^2= 0,8595	Hb^A	0.0850	0.8056	0.1944	0.1559
		Hb^B	0.9150			
	Tsurcana χ^2= 4,8246	Hb^A	0.1667	0.6527	0.3473	0.2785
		Hb^B	0.8333			
	Botosani Karakul χ^2= 0,4315	Hb^A	0.0667	0.8444	0.1556	0.1248
		Hb^B	0.9333			
Transferrin system	Palas Merino χ^2= 62,2309**	Tf^I	0.0000	0.0785	0.9215	0.7391
		Tf^A	0.3840			
		Tf^G	0.0010			
		Tf^B	0.0260			
		Tf^C	0.0930			
		Tf^M	0.2290			
		Tf^D	0.2290			
		Tf^E	0.0330			
		Tf^P	0.0050			
	Tsigai χ^2= 40,3123	Tf^I	0.0080	0.0185	0.9814	0.7871
		Tf^A	0.2580			
		Tf^G	0.0070			
		Tf^B	0.2420			
		Tf^C	0.0910			
		Tf^M	0.1210			
		Tf^D	0.2580			
		Tf^E	0.0070			
		Tf^P	0.0080			
	Tsurcana χ^2= 61,7576***	Tf^I	0.0000	0.0396	0.9604	0.7703
		Tf^A	0.2200			
		Tf^G	0.0200			
		Tf^B	0.2250			
		Tf^C	0.3400			
		Tf^M	0.1150			
		Tf^D	0.0500			
		Tf^E	0.0300			
		Tf^P	0.0000			
	Botosani Karakul χ^2= 57,4509***	Tf^I	0.0000	0.3182	0.6818	0.5468
		Tf^A	0.0278			
		Tf^G	0.0000			
		Tf^B	0.3250			
		Tf^C	0.5889			
		Tf^M	0.0278			
		Tf^D	0.0222			
		Tf^E	0.0083			
		Tf^P	0.0000			
Albumin system	Palas Merino χ^2= 66,4717***	Alb^F	0.0455	0.8914	0.1086	0.0871
		Alb^S	0.9545			
	Tsigai χ^2= 0,302	Alb^F	0.0375	0.9098	0.0902	0.0724
		Alb^S	0.9625			
	Tsurcana χ^2= 0,00	Alb^F	0.0000	1.0000	0.0000	0.0000
		Alb^S	1.0000			
	Botosani Karakul χ^2= 0,00	Alb^F	0.0000	1.0000	0.0000	0.0000
		Alb^S	1.0000			
Amylase system	Palas Merino χ^2= 0,00	Am^B	1.0000	1.0000	0.0000	0.0000
		Am^C	0.0000			
	Tsigai χ^2=1,4014	Am^B	0.9200	0.8160	0.1840	0.1476
		Am^C	0.0800			
	Tsurcana χ^2= 0,00	Am^B	1.0000	1.0000	0.0000	0.0000
		Am^C	0.0000			
	Botosani Karakul χ^2= 0,00	Am^B	1.0000	1.0000	0.0000	0.0000
		Am^C	0.0000			
Potassium system	Palas Merino	K^L	1.0000	1.0000	0.0000	0.0000
		K^h	0.0000			
	Tsigai	K^L	0.9350	0.8481	0.1519	0.1219
		K^h	0.0650			
	Tsurcana	K^L	0.4804	0.3760	0.6240	0.5005
		K^h	0.5196			
	Botosani Karakul	K^L	0.0787	0.8187	0.1813	0.1454
		K^h	0.9213			

Table 2. Coefficients of informational energy (*e*), genetic diversity (*d*) and heterozygosity (*Ht*) at the loci of immunogenetic systems in the Romanian sheep breeds

Blood group system	Blood factor	Breed	Allelic frequency				e	d	Ht
			dominant allele		recessive allele				
System A	Aa	Palas Merino	Aa^+	0.3505	Aa^-	0.6495	0.4309	0.5691	0.4564
		Tsigai		0.3390		0.6610	0.4398	0.5602	0.4493
		Tsurcana		0.9458		0.0542	0.8718	0.1282	0.1028
		Botosani Karakul		0.5793		0.4207	0.3907	0.6093	0.4886
System B	Bb	Palas Merino	Bb^+	0.4261	Bb^-	0.5739	0.3886	0.6113	0.4903
		Tsigai		0.2812		0.7188	0.4945	0.5053	0.4053
		Tsurcana		0.6910		0.3090	0.4662	0.5338	0.4281
		Botosani Karakul		0.5488		0.4512	0.3809	0.6190	0.4965
	Bc	Palas Merino	Bc^+	0.2100	Bc^-	0.7900	0.5852	0.4147	0.3326
		Tsigai		0.1215		0.8785	0.7332	0.2668	0.2140
		Tsurcana		0.3422		0.6578	0.4372	0.5627	0.4513
		Botosani Karakul		0.3060		0.6940	0.4691	0.5309	0.4258
	Bd	Palas Merino	Bd^+	0.1100	Bd^-	0.8900	0.7552	0.2447	0.1963
		Tsigai		0.2112		0.7888	0.5835	0.4165	0.3340
		Tsurcana		0.2766		0.7234	0.4998	0.5002	0.4012
		Botosani Karakul		0.4002		0.5998	0.3984	0.6016	0.4813
	Bf	Palas Merinos	Bf^+	0.3090	Bf^-	0.6910	0.4662	0.5338	0.4281
		Tsigai		0.2879		0.7121	0.4875	0.5125	0.4111
		Tsurcana		0.8056		0.1944	0.6085	0.3915	0.3140
		Botosani Karakul		0.4879		0.5121	0.3754	0.6246	0.5010
	Bg	Palas Merino	Bg^+	0.0890	Bg^-	0.9110	0.7973	0.2027	0.1626
		Tsigai		0.1898		0.8102	0.6156	0.3844	0.3083
		Tsurcana		0.2801		0.7199	0.4959	0.5041	0.4043
		Botosani Karakul		0.3729		0.6271	0.4154	0.5846	0.4689
	Bi	Palas Merino	Bi^+	0.2730	Bi^-	0.7270	0.5038	0.4962	0.3979
		Tsigai		0.2595		0.7405	0.5196	0.4804	0.3853
		Tsurcana		0.3508		0.6492	0.4306	0.5693	0.4566
		Botosani Karakul		0.3060		0.6940	0.4691	0.5309	0.4258
System C	Ca	Palas Merino	Ca^+	0.0590	Ca^-	0.9410	0.8612	0.1388	0.1113
		Tsigai		0.0831		0.9169	0.8095	0.1905	0.1528
		Tsurcana		0.5329		0.4671	0.3777	0.6223	0.4991
		Botosani Karakul		0.4369		0.5631	0.3849	0.6150	0.4933
	Cb	Palas Merino	Cb^+	0.6690	Cb^-	0.3310	0.4464	0.5536	0.4440
		Tsigai		0.5303		0.4697	0.3773	0.6227	0.4994
		Tsurcana		0.7634		0.2366	0.5484	0.4515	0.3621
		Botosani Karakul		0.3107		0.6893	0.4646	0.5354	0.4294
System D	Da	Palas Merino	Da^+	0.1430	Da^-	0.8570	0.6936	0.3064	0.2457
		Tsigai		0.2083		0.7917	0.5877	0.4123	0.3306
		Tsurcana		0.3921		0.6079	0.4041	0.5959	0.4779
		Botosani Karakul		0.3593		0.6407	0.4245	0.5755	0.4616
System M	Ma	Palas Merinos	Ma^+	0.2020	Ma^-	0.7980	0.5970	0.4030	0.3232
		Tsigai		0.6680		0.3320	0.4456	0.5544	0.4445
		Tsurcana		0.5969		0.4031	0.3985	0.6015	0.4824
		Botosani Karakul		0.4646		0.5354	0.3781	0.6219	0.4987
System R-O	R	Palas Merino	R^+	0.3040	R^-	0.6960	0.4710	0.5290	0.4242
		Tsigai		0.1780		0.8220	0.6342	0.3658	0.2934
		Tsurcana		0.2546		0.7454	0.5255	0.4744	0.3805
		Botosani Karakul		0.2997		0.7003	0.4753	0.5247	0.4208
	O	Palas Merino	O^+	0.2830	O^-	0.7170	0.4927	0.5073	0.4068
		Tsigai		0.2690		0.7310	0.5084	0.4916	0.3943
		Tsurcana		0.5487		0.4513	0.3809	0.6191	0.4965
		Botosani Karakul		0.3340		0.6660	0.4439	0.5561	0.4460

Table 3. Coefficients of informational energy (e), genetic diversity (d) and heterozygosity (Ht) at the loci of molecular-genetic systems in the Romanian sheep breeds

Molecular-genetic system	Breed	Allele	Allelic frequency	e	d	Ht
β-Lactoglobulin system	Palas Merino χ^2= 64,5945***	LGB^A	0.5550	0.3826	0.6174	0.4952
		LBG^B	0.4450			
	Tsigai χ^2= 9,7537**	LGB^A	0.5420	0.3794	0.6206	0.4977
		LBG^B	0.4580			
	Tsurcana χ^2= 1,9213	LGB^A	0.6870	0.4624	0.5376	0.4311
		LBG^B	0.3130			
	Botosani Karakul χ^2= 8,9059**	LGB^A	0.7350	0.5131	0.4869	0.3905
		LBG^B	0.2650			
Calpastatin system	Palas Merino χ^2= 4,9392	$CAST^A$	0.7969	0.5954	0.4046	0.3245
		$CAST^B$	0.2031			
	Tsigai χ^2= 2,2879	$CAST^A$	0.7684	0.5551	0.4449	0.3568
		$CAST^B$	0.2316			
	Tsurcana χ^2= 3,6612	$CAST^A$	0.8624	0.7033	0.2967	0.2379
		$CAST^B$	0.1376			
	Botosani Karakul 71.9659***	$CAST^A$	0.8468	0.6757	0.3243	0.2601
		$CAST^B$	0.1532			
α_s1-casein system	Palas Merino χ^2= 0,00	Cn^A	0.0000	1.0000	0.0000	0.0000
		Cn^C	1.0000			
	Tsigai χ^2= 0,00	Cn^A	0.0000	1.0000	0.0000	0.0000
		Cn^C	1.0000			
	Tsurcana χ^2= 0,00	Cn^A	0.0000	1.0000	0.0000	0.0000
		Cn^C	1.0000			
	Botosani Karakul χ^2= 0,00	Cn^A	0.0000	1.0000	0.0000	0.0000
		Cn^C	1.0000			
Prion system	Palas Merino χ^2 = 4.93	ARR	0.4108	0.2768	0.7232	0.5801
		ARQ	0.5000			
		AHQ	0.0270			
		ARH	0.0297			
		VRQ	0.0325			
	Tsigai χ^2 = 5.12	ARR	0.4189	0.3191	0.6809	0.5455
		ARQ	0.5279			
		AHQ	0.0080			
		ARH	0.0199			
		VRQ	0.0253			
	Tsurcana χ^2 = 10,29	ARR	0.3755	0.2645	0.7355	0.5896
		ARQ	0.5143			
		AHQ	0.0306			
		ARH	0.0082			
		VRQ	0.0714			
	Botosani Karakul χ^2 = 29,74***	ARR	0.4025	0.3384	0.6616	0.5306
		ARQ	0.5539			
		AHQ	0.0000			
		ARH	0.0436			
		VRQ	0.0000			
SLS system	Palas Merino χ^2= 0,00	FGFR3	1.0000	1.0000	0.0000	0.0000
		$FGFR3^+$	0.0000			
	Tsigai χ^2= 0,00	FGFR3	1.0000	1.0000	0.0000	0.0000
		$FGFR3^+$	0.0000			
	Tsurcana χ^2= 0,00	FGFR3	1.0000	1.0000	0.0000	0.0000
		$FGFR3^+$	0.0000			
	Botosani Karakul χ^2= 0,00	FGFR3	1.0000	1.0000	0.0000	0.0000
		$FGFR3^+$	0.0000			
Booroola system	Palas Merino χ^2= 0,00	FecB	1.0000	1.0000	0.0000	0.0000
		$FecB^+$	0.0000			
	Tsigai χ^2= 0,00	FecB	1.0000	1.0000	0.0000	0.0000
		$FecB^+$	0.0000			
	Tsurcana χ^2= 0,00	FecB	1.0000	1.0000	0.0000	0.0000
		$FecB^+$	0.0000			
	Botosani Karakul χ^2= 0,00	FecB	1.0000	1.0000	0.0000	0.0000
		$FecB^+$	0.0000			
Inverdale system	Palas Merino χ^2= 0,00	FecX	1.0000	1.0000	0.0000	0.0000
		$FecX^+$	0.0000			
	Tsigai χ^2= 0,00	FecX	1.0000	1.0000	0.0000	0.0000
		$FecX^+$	0.0000			
	Tsurcana χ^2= 0,00	FecX	1.0000	1.0000	0.0000	0.0000
		$FecX^+$	0.0000			
	Botosani Karakul χ^2= 0,00	FecX	1.0000	1.0000	0.0000	0.0000
		$FecX^+$	0.0000			

The 3/1 representation ratio between the two calpastatin alleles in Palas Merino and Tsigai causes a moderate genetic diversity being slightly below 0.50. The gap widening between the $CAST^A$ and $CAST^B$ allele frequencies leads to decreasing of calpastatin diversity too in the other two breeds (around 0.30).

In the prion system, the larger number of alleles at the PrP locus also influences the diversity by its increasing. In Palas Merino, Tisgai and Tsurcana, where all five prion alleles can be found, the genetic diversity degree is quite high; the differences among the three breeds come from the distribution way of these alleles. In Tsurcana, where the five alleles are more evenly spread, there is the richest diversity (about 0.74), as well as in Palas Merino (0.72). In Tsigai, because the alleles are less evenly spread a decrease of prion diversity occurs. In the Botosani Karakul breed, both the presence of only three alleles and their distribution unevenness have the effect a lower prion diversity coefficient than in the other three breeds although its decreasing is not significant.

Although the α_s1-casein, ovine hereditary chondrodysplasia (SLS), Booroola and Inverdale systems are biallelic systems, they are monotypic at respective loci in Romanian sheep breeds. In each of these four systems, only one allele is expressed, respectively Cn^C, FRFG3, FecB and FecX, their codominant missing, respectively Cn^A, $FRFG3^+$ $FecB^+$ and $FecX^+$. The polymorphism lack at these loci generates maximum informational energies, their counterpart being the complete absence of genetic diversity in these molecular systems.

Complex genetic diversity

To get an overview of the genetic diversity quantified within the multiple and associated systems, breeds, etc. (complex genetic diversity - d_t), the synthetic indicator is used, namely the complex informational energy (e_t).

Complex genetic diversity on associated system types

Within the <u>*biochemical-genetic systems*</u>, the large number of alleles and their spread range in the transferrin system generate extremely low informational energies. As a result, the genetic diversity calculated on all breeds throughout transferrin system is very high (about 0.89). In haemoglobin and kalium systems of biallelic type, in which the polymorphism has a middle level, the genetic diversity is moderate (0.27 in haemoglobin system, respectively 0.24 in potassium system). In the albumin and amylase systems, where most breeds are monotypic at the respective loci or in those where there is polymorphism but this is very narrow, the genetic diversity is extremely low (only 0.05). In relation to these coefficients, the total genetic-biochemical systemic diversity is moderate (0.30) (Figure 1).

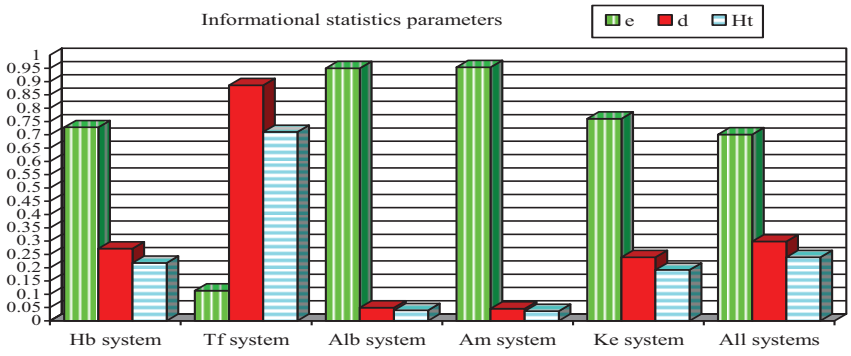

Figure 1. Coefficients of complex informational energy (***e$_t$***), complex genetic diversity (***d$_t$***) and heterozygosity (***Ht***) in the biochemical-genetic systems in the Romanian sheep breeds

Within the <u>*immunogenetic systems*</u> there is a relatively uniform distribution of the two types of alleles (dominant and recessive) at the level of all blood group factors. Even if there are differences in relation to the frequencies of the two allele types, the gaps between them are not exaggerated. For this fact, for most blood factors, the diversity is of middle level, its

framing range being between 0.45 and 0.55. Only at the Ca factor level the variability is lower, however its value have to taken into consideration (0.39). The variability at Bf and Bi blood factors is circumscribed at the 0.5 limit. Higher values than this threshold are achieved by Bb, Cb, Ma and O factors. At the level of factors Aa, Bc, Bd, Bg, Da and R, the immunogenetic variability records scores below the 0.50 median limit. Given the values of these coefficients between the two informational-statistical parameters there is an almost unitary ratio, with a weaker surplus for the informational energy (0.51). As a result, the total immunogenetic diversity is in close proximity to the median threshold (about 0.49) (Figure 2).

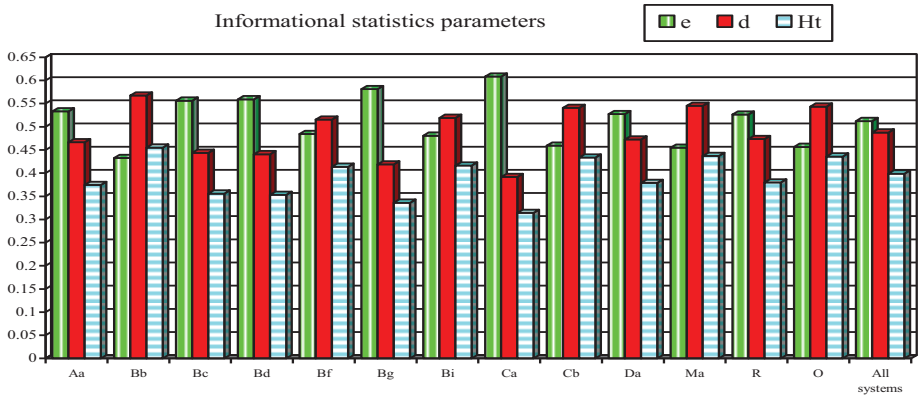

Figure 2. Coefficients of complex informational energy (***e_t***), complex genetic diversity (***d_t***) and heterozygosity (***Ht***) in the immunogenetic systems in the Romanian sheep breeds

The most variable system within the *molecular-genetic systems* is the polyallelic system of prion protein. The five alleles as well as their dispersion in prion panel lead to a high enough diversity (0.70). Within the other six systems of biallelic type, only at LGB and CAST loci there is genetic polymorphism. In the β-lactoglobulin system, due to a more balanced ratio between the two alleles there is a considerable diversity, above the average limit, measuring about 0.56. On the other hand, in the calpastatin system there is a more obvious representation gap of $CAST^A$ and $CAST^B$ alleles, which makes the diversity to be more moderate, too (about 0.37). In the α_s1-casein, hereditary chondrodysplasia and fecundity gene (Booroola and Inverdale) systems the Romanian sheep breeds being monomorphic there is not genetic diversity. For all these reasons, the total molecular-genetic diversity is low (about 0.23) (Figure 3).

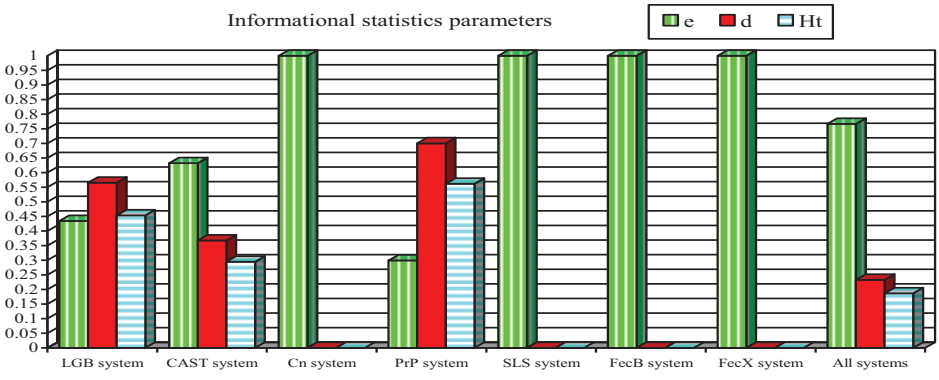

Figure 3. Coefficients of complex informational energy (***e_t***), complex genetic diversity (***d_t***) and heterozygosity (***Ht***) in the molecular-genetic systems in the Romanian sheep breeds

Complex genetic diversity on sheep breeds

An informational statistics analysis of the four Romanian sheep breeds on the three types of genetic systems leads to as real as possible quantification of genetic diversity.

Within the *biochemical-genetic systems* the most diverse breed (of middle level) is Tsurcana (0.39) and Botosani Karakul is the breed with the lowest diversity degree (0.20). The moderate diversity of Tsigai (0.32) and Palas Merino (0.28) is situated in this variability range. Overall the Romanian sheep breeds the biochemical-genetic diversity is of moderate level (0.30) (Figure 4).

Within the *immunogenetic systems* the breed with the greatest diversity is Botosani Karakul in which the value of this coefficient is considerable (0.58). The Tsurcana breed performs a diversity of middle level (0.50). In the other two breeds the genetic diversity is below the median threshold, but it has values to be taken into consideration, 0.44 in Tsigai and 0.42 in Palas Merino. Because of these issues, the total immunogenetic diversity for the four Romanian sheep breeds has a medium level (0.49) (Figure 5).

Within the *molecular-genetic systems*, among the four Romanian sheep breeds no significant differences are recorded, the coefficients of this informational-statistical parameter ranging in a narrow enough range from 0.21 in Botosani Karakul to 0.25 in Palas Merino and Tsigai; the Tsurcana diversity (about 0.22) is placed between these limits. On the whole of ovine species from Romania, the molecular-genetic diversity is relatively low (0.23) (Figure 6).

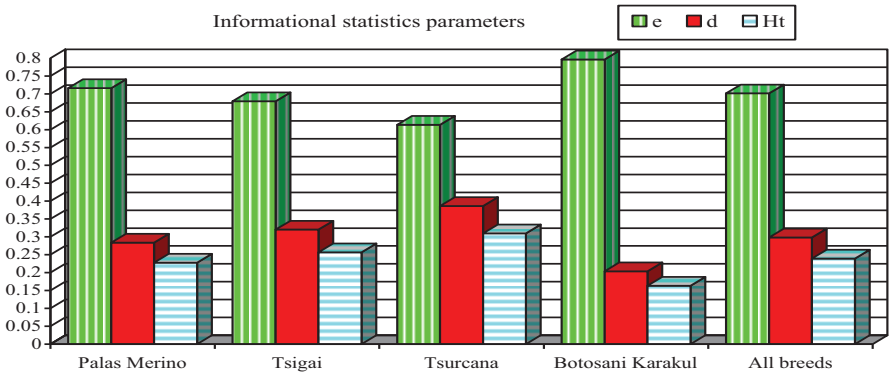

Figure 4. Coefficients of complex informational energy (e_t), complex genetic diversity (d_t) and heterozygosity (***Ht***) within the biochemical-genetic systems on each Romanian sheep breed

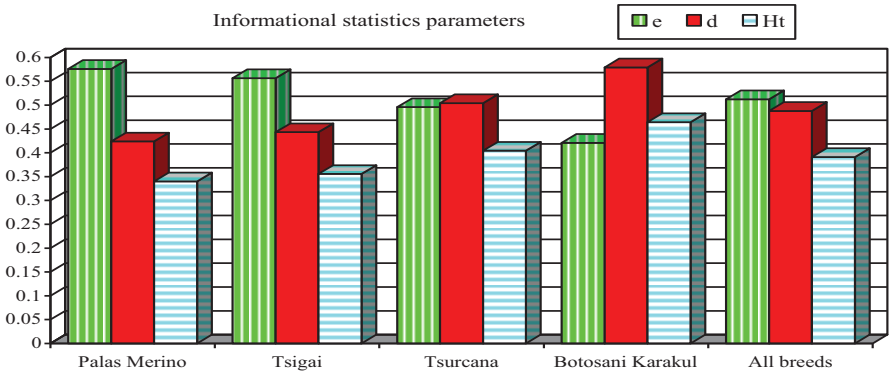

Figure 5. Coefficients of complex informational energy (e_t), complex genetic diversity (d_t) and heterozygosity (***Ht***) within the immunogenetic systems on each Romanian sheep breed

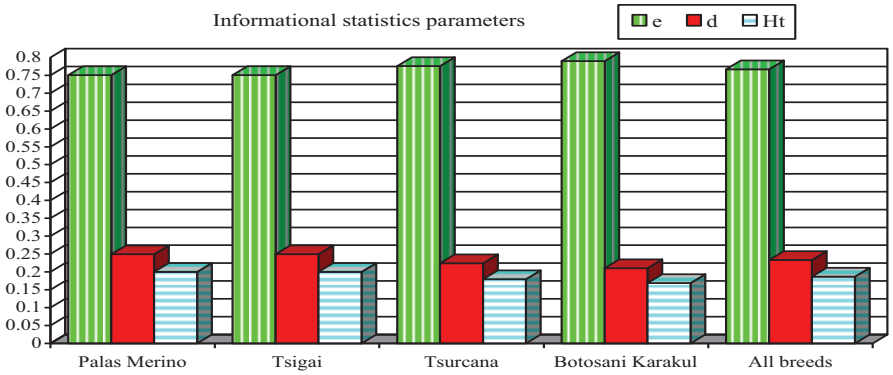

Figure 6. Coefficients of complex informational energy (e_t), complex genetic diversity (d_t) and heterozygosity (***Ht***) within the molecular-genetic systems on each Romanian sheep breed

Summing *all genetic diversity coefficients* of the three types of polymorphic systems, it is clear that Tsurcana is the most diverse breed, close to the medium level (0.37). A moderate diversity is met in the other three breeds with very small differences among breeds, 0.32 in Palas Merino, 0.33 in Botosani Karakul and 0.34 in Tsigai. On all four Romanian sheep breeds the total genetic diversity recorded a moderate level (0.34) (Figure 7).

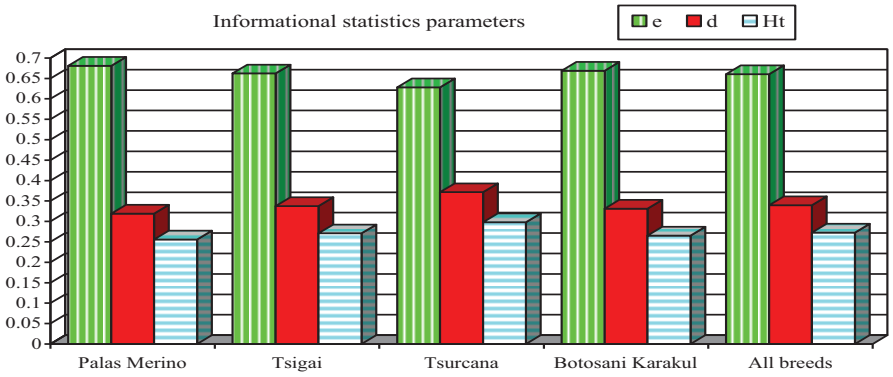

Figure 7. Coefficients of complex informational energy (e_t), complex genetic diversity (d_t) and heterozygosity (***Ht***) within all genetic systems on each Romanian sheep breed

The coefficient value of informational energy or genetic diversity is a measure of the *polymorphism degree*, both of the genetic systems and of the sheep breeds at all loci levels.

The polyalellic systems are the most allotropic, the highest polymorphism level having firstly the transferrin system and then the prionic system. In the biallelic systems, the polymorphism occurs where both alleles are expressed, either in codominant status or in dominance to recessiveness status. The polymorphism is marked to some of them, such as in the β-lactoglobulin system and at most blood group factors. Within some systems the polymorphism degree is moderate, such as in haemoglobin, potassium, calpastatin and C systems, at the Ca factor level. The polymorphism is barely perceptible in others of them (albumin in Palas Merino and Tsigai and amylase in Tsigai). The biallelic systems in which only one allelic type is expressed are characterized by genetic monomorphism: albumin system in Tsurcana and Botosani Karakul, amylase system in Palas Merino Tsurcana and Botosani Karakul, kalium system in Palas Merino and α_s1-casein, SLS, Booroola and Inverdale systems in all sheep breeds.

In the biochemical-genetic respect, the

Tsurcana sheep presents the highest polymorphism degree and the narrowest polymorphism is found in Botosani Karakul. The polymorphism of biochemical-genetic structures of Palas Merino is slightly more emphasized than of the Tsigai.

The immunogenetic structures are highly polymorphic in all sheep breeds, but Botosani Karakul is the most endowed with erythrocyte antigens and Palas Merino is the poorest from the immunogenetically point of view.

The molecular-genetic polymorphism is limited enough in all Romanian sheep breeds and the differences among breeds are minor: the Palas Merino and Tsigai breeds are slightly more polymorphic than Tsurcana and especially than Botosani Karakul.

In terms of all structures with multiple molecular forms, Tsurcana is the most polymorphic breed, much more polymorphic than Botosani Karakul or Tsigai and in particular towards the Palas Merino. But throughout the ovine species from Romania the genetic polymorphism is moderate.

The *heterozygosity degree* of a taxonomic entity for different characters is the true measure of genetic variability. In this regard, it is noted that there is perfect concordance between the genetic diversity and heterozygosity status in the Romanian sheep breeds at the level of all genetic system loci. The differences between these two informational statistics parameters are derived from their values, the heterozygosity being reduced steadily in all situations, with about 22%. Thus, a graphical representation of the two genetic parameters is enlightening as regards the identical allure of their curves. For illustration, we present only the diagram concerning the relationship between diversity and heterozygosity on all genetic systems in the four Romanian sheep breeds (Figure 8).

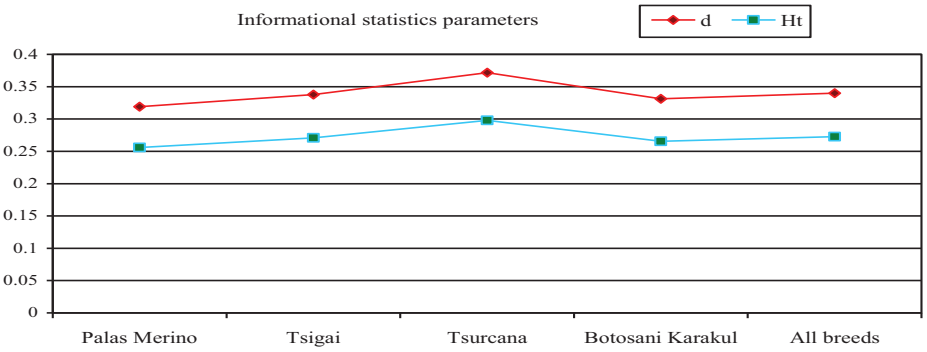

Figure 8. Distributional curves of genetic diversity (**d**) and heterozygosity (**Ht**) within all genetic systems on each Romanian sheep breed

Discussing in cybernetic terms, the values of the two indicators of informational statistics express the *systemic organization degree*, an opposite concept to *entropy* (*H*); when the informational energy is lower and the genetic diversity is more significant, then the organization degree of the system is higher; on the contrary, when the informational energy increases and genetic diversity decreases, then the entropy increases in system (the disorganization degree becomes more emphasized). The highest organization degree is met in the systems of biallelic type, but in which only one allele type is expressed (amylase system in Palas Merino, Tsurcana and Botosani Karakul, potassium system in Palas Merino and α_s1-casein, SLS, Booroola and Inverdale systems in all sheep breeds). In the albumin (in Palas Merino and Tsigai) and amylase (in Tsigai) systems the first signs of entropy appear. The disorganization degree increases in haemoglobin, kalium and calpastatin systems and becomes more emphasized in β-lactoglobulin and blood group systems. In the prionic system and especially in the transferrin one it comes out the highest degree of systemic entropy. From this perspective, the breeds can have different levels of entropy with particular reference to each genetic system or system association, but overall the genetic

structures all breeds have roughly the same degree of systemic organization.
From this analysis, it should be mentioned that the polymorphic panel of hereditary heritage of some biological entities acquires greater accuracy if as many genetic systems participate to the construction of the informational-statistical edifice and as they are more diversified like the number of alleles and their distribution.
Determining as correct as possible the genetic diversity structures of sheep breeds contributes to the enriching of cognitive status relating to the genetic variability and genetic resources within this species. This inventory of genetic resources is necessary for development of improvement programs of sheep breeds of economic interest and perfecting of the existing ones, but also to initiate and implement the programs to protect and preserve the genetic potential of breeds in a vulnerability status (Cohen, 1999). The conservation and enhancement of genetic diversity is a key condition in performing the farm animal breeding programs, the diversity becoming, justifiably, one of the most evoked concepts in the economical and environmental policies (Gandini et al., 2007). There are also situations when the genetic diversity increasing at some loci can be dangerous. This issue can be met in the case of genetic diseases and those with hereditary predisposition, by increasing the incidence of lethal alleles, which can produce considerable economic losses and harm effects on animal and human health (Álvarez et al., 2009; Hrincă, 2016b, 2016c).

CONCLUSIONS

Using the informational energy (*e*) the genetic diversity (*d*) was quantified in four Romanian sheep breeds (Merino Palas, Tsigai, Tsurcana and Botosani Karakul) within three types of genetic systems: biochemical-genetic (haemoglobin, transferrin, albumin, amylase, blood potassium), immunogenetic (blood groups A, B, C, D, M, R-O) and molecular-genetic (α_s1-casein, β-lactoglobulin, SLS, Booroola, Inverdale).
The calculus algorithm for determining the size of the two informational-statistical indicators was based on frequencies of 70 alleles: 17 alleles of biochemical-genetic systems, 36 alleles of immunogenetic systems and 17 alleles of molecular-genetic systems.
The genetic diversity level is an informational-statistical function provided by the allele number, their frequencies, the ratio among them within the systems and the polymorphism degree of genetic systems.
The highest diversity occurs in the polyallelic systems (transferrin and prion protein).
In the other systems, of biallelic type, the genetic diversity occurs where both allele types, either in codominant status or in complete dominance relation, are present in the panels of respective systems; in the systems in which the two allele types are more evenly spread, the genetic diversity is more relevant (β-lactoglobulin and blood group systems) and in the systems where the frequency gap gets larger it comes out a decrease of diversity (haemoglobin, blood potassium, calpastatin); in the albumin and amylase systems (in some breeds), where one of allele is prevailing, the diversity is very low.
In the monomorphic systems in which only one allele type is expressed (albumin and amylase systems in some breeds and α_s1-casein, hereditary chondrodysplasia protein and FecB and FecX gene fecundity systems in all breeds), the genetic diversity is completely missing.
Among the Romanian sheep breeds there are larger or smaller differences regarding the genetic diversity levels when the analysis takes into account each system or even when diversity is measured on the three system types (biochemical-genetic, immunogenetic and molecular-genetic), but summing all systems the differences among breeds are small and unsignificant regarding the genetic diversity as a whole.
The informational-statistical quantification of genetic diversity in sheep is a very useful tool in breeding programs of economic interest breeds and for preservation of the vulnerable ones.

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