Polymorphism of Blood Serum Protein- Transferrin, Ceruloplasmin, Albumin- in Sheep of Karabakh Breed and the Relationship with the Natural Resistance Towards Epizootic Aphtae

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Abstract
New genetic methods are currently used in selection activities of agricultural animals, based on polymorph genetic determination systems, which, serving as genetic markers, enable studying animal genealogy, alelofon, genotypes. The current study is targeted at the genetic characteristics of Karabakh sheep breed, based on the loci of transferrin (Tf), ceruloplasmin (Cp) and albumin (Al), as well as the relationship with their resistance towards disease epizootic aphtae in order to use the acquired results in selection activities as a test. The result of our research indicate that the loci of Tf, Cp and Al proteins of blood serum in the sheep of Karabakh breed are polymorphic consisting of A, B, C alleles. Tf locus displays eight, and Cp and Al six genotypes. The homozygosity rate of the loci makes 20; 40 and 30%. In case of epizootic aphtae disease virus circulation, without any characteristic clinical symptoms, high resistance has been observed in the animals with TfAC, TfBB, TfBC, CpAC, CpBC, AlAB, AlBC genotypes, the titer of nonstructural proteins in these animals has made 2.15; 1.6; 5.4; 3.6; 1.4; 5.2 and 5.2%. The results can be used in the selection activities in sheep breeding.

Keywords: Albumin, allele, ceruloplasmin, polymorphism, transferrin.

1. Introduction

Armenia is situated within the Caucasian territory which is one of the most important, richest and vulnerable areas among the 25 hot spots of the world bio-diversity. Related to natural and climatic conditions, Armenia not only has rich fauna, but it is also considered to be the generation source for a number of farm animals of different breeds, which have been raised for centuries both through the popular and scientific selection methods. For the accounting, preservation and efficient use of genetic resources of agricultural animals the specification of genetic structures of different populations, allele funds and genotypes are very important.

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In view of this we are conducting some activities aimed at the classification of a number of valuable congeners according to their genotypes bred in Armenia using the protein polymorphism and micro-satellite DNA as genetic markers [1,2].
In the current research the genetic characteristics of the sheep of Karabakh breed according to the loci of transferrin (Tf), ceruloplasmin (Cp) and albumin (Al) and the relationship with their natural resistance towards epizooticae aphtae are introduced, in order to use the acquired results in the sheep selection activities as a test [3].

2. Materials and methods

The sheep of Karabakh breed is bred in the Syunik region of RA and in Nagorno Karabakh Republic. It has been established through the popular

selection and has got dairy, meat and wool directions.

The blood sampling for the sheep of Karabakh breed was conducted from the rural farm of Shaqe community at Syunik region.

The blood samples were taken from the jugular vein of 20 heads of sheep of the age of 3 through the vacuum test tube containing gel activator.

Coagulated blood was subjected to centrifugation (5 minutes, 6000 rpm) for the separation of the blood serum.

Through electrophoresis the polymorphism of transferrin (Tf), ceruloplasmin(Cp) and albumin (Al) proteins was investigated.

Electrophoresis has been conducted through Davis method [4], with «Multigel-long» phoresis apparatus of "Biometra" brand, on 10% polyacrylamide gel according to the data of the Table 1.

Table 1. Respective terms for electrophoresis of blood serum Tf, Cp and Al loci

Locus	Gel, %	Gel length, cm	Sample titer	Buffer		Power voltage, V	Phoresis duration, hour
				Gel	Electrode		
Tf	10	12	1:2	0.05M tris HCL pH 8.8	0.016 M tris glycine pH 8.7	280	3
Cp	10	12	1:1	0.18 M tris HCL pH 8.8	0.016 M tris Borate pH 9.0	290	2.5
Al	10	10	1:5	0.18 M tris HCL pH 8.8	0.005 M tris glycine pH 8.9	220	4

After the phoresis termination, the gel was fixed in the ethanol, acetous acid and distilled water (40:10:60) solution for 60 minutes and then was dyed with Coomassie dye G-250 for 30-60 minutes and washed with washing buffer thrice.

The genotype and allele frequency was determined by the following formula:

$$P_i = \frac{n_i}{N}$$

Where P_i-is the frequency of i allele

n_i-is the number of allele-bearing animals

N-is the total number of researched animals

For the individual loci the share of genotypes homozygosity was determined upon the principle of the common ratio of the total number of researched animals.

For the first time we have set a task to study the polymorphic proteins and the availability of structural and non-structural proteins of the blood serum in the sheep of Karabakh breed with various genotypes (according to Tf, Cp, Al loci), which has been carried out by means of immuno-enzyme analyses (ELISA-At). Such tests enable to disclose the circulation of epizooticae aphtae virus in the organisms of sensitive animals.

3. Results and discussion

The main issue of bio-chemistry genetics is the description of homologous loci, which enables not only to clarify the genetic structure of different populations but also to disclose the current issues in the given area, such as, for example, the animal resistance towards the diseases.

It is known that the transferrin in the sheep is regulated by five co-dominant alleles of autosomal locus [5].

As a result of our study three of them have been discovered- TFA, TFB, TFC,- out of which the highest frequency is observed in TFA (0.55). The occurrence frequency of TFB, TFC alleles is twice as low as that of TFA allele (table 2). Besides the TFA allele has been manifested by 3 genotypes: TFAA (0.55), TFAB (0.10) and TFAC (0.40). As we can see from the same table, the frequency of TFAC genotype is the highest, while the TFAA genotype has the lowest occurrence frequency.

TFB allele has been manifested with two genotypes: homozygote TFBB (0.10) and heterozygote TFBC (0.05).

TFC allele has formed three genotypes: TFCC (0.05), TFCD (0.15) and TFCE (0.05). The rate of locus homozygosity has made 20%.

Table 2. Genetic characteristics of the sheep of Karabakh breed according to Tf, Cp and Al loci

Locus	n	Frequency Pi											Homozygosity rate according to individual loci
		Genotypes %								Alleles			
		AA	AB	AC	BB	BC	CC	CD	CE	A	B	C	
Tf	20	0.05	0.10	0.40	0.10	0.10	0.05	0.15	0.05	0.55	0.20	0.25	20
Cp	20	0.10	0.25	0.10	0.05	0.25	0.25	-	-	0.45	0.30	0.25	40
Al	20	0.05	0.15	0.20	0.10	0.35	0.15	-	-	0.40	0.45	0.15	30

The results are interesting in case of ceruloplasmin locus. Our research has disclosed that it is polymorphic, as well consisting of CPA, CPB, CPC alleles, the occurrence frequency of which is equal to 0.45; 0.30 and 0.25 respectively (table 2). CPA allele is manifested with 3 genotypes: homozygote CPAA (0.10) and heterozygote CPAB (0.25) and CPAC (0.10). The homozygosity rate of the locus is rather high; it makes 40%.

As to the albumin locus (AL) , it can be definitely stated, that it is also polymorphic consisting of rapidly migrated ALA (0.40), ALB (0.45) with average translocation speed and slowly migrated ALC (0.15) alleles.

Out of 12 genotype variants 6 variants have been discovered in the researched sheep group: AL AA, AB, AC, BB, BC, CC (table 2). Besides, ALA allele has been manifested by homozygote AL AA (0.10) and heterozygote AL AB (0.25) and AL AC (0.10) genotypes. ALB allele is characterized by two genotypes: AL BB (0.05) homozygote and AL BC (0.25) heterozygote.

Concerning the ALC allele, it should be mentioned, that it has formed only one homozygote genotype ALCC (0.15). The homozygosity rate of the locus is rather high and it makes 30%.

It is obvious that the intensification of different branches of animal husbandry is directly related to the prevention and treatment of the diseases. Among different diseases, the microbial ones take great place and the issues on the increase of the animal genetic resistance is highly prioritized nowadays; however, the research achives carried out in this direction have shown that only in this way, without excessive costs, the animals can be protected from various diseases.

The genetically related immuno-resistance of agricultural animals is a global issue today, and the disclosure of genetic markers for the attribute having polygene relationship is very topical [6-8].

At the same time, it should be mentioned that in similar conditions the titer of non-structural proteins is very low or lacks at all in animals, whith high immunity.

The data of Table 3 show that all researched animals had been vaccinated with anti-foot-and-mouth disease vaccine, since the inhibition titer of structural proteins were higher than 50%:

Table 3. Genetic structure of the sheep of Karabakh breed according to the loci of Tf, Cp and Al, relationship with structural and nonstructural proteins

Locus/ anti-bodies		n	Genotype							
		20	AA	AB	AC	BB	BC	CC	CD	CE
Tf	structural		67	75	81	77	83	59	69	74
	nonstructural		9.8	10.2	2.1	1.6	5.4	7.5	12.2	11.8
Cp	structural		59	75	71	68	54	89	-	-
	nonstructural		8.7	10.3	3.6	5.8	1.4	7.4	-	-
Al	structural		66	72	51	89	74	85	-	-
	nonstructural		9.5	5.2	7.5	9.4	5.2	-	-	

As to nonstructural proteins, which appear in the organism in case of virus availability, it should be mentioned that the virus titer is the lowest in the sheep with Tf- AC (2.15), BB (1.6), BC (5.4); Cp-AC (3.6), BC (1.4) and AL- AB (5.2) and BC (5.2) genotypes. This indicates that in case of circulation of foot-and-mouth disease virus, without any characteristic clinic, the animals with the above mentioned genotypes demonstrate natural high resistance towards the mentioned disease. All these can be used as genetic markers in the process of the branch selection.

4. Conclusions

The analyses of the acquired facts resuling from the experiment enable to draw the following conclusions:

1. The relevant loci of transferrin, ceruloplasmin and albumin proteins of the blood serum in the sheep of Karabakh breed are polymorphic consisting of A, B, C alleles. Transferrin locus displays eight, and ceruloplasmin and albumin six genotypes. The homozygosity rate of the loci makes 20; 40 and 30 % respectively.
2. In case of epizooticae aphtae virus circulation, without any characteristic clinical symptoms, natural high resistance has been observed in the animals with TfAC, TfBB, TfBC, CpAC, CpBC, AlAB, AlBC genotypes, the titer of nonstructural proteins in these animals has made 2.15; 1.6; 5.4; 3.6; 1.4; 5.2 and 5.2% respectively.

Proposals: The acquired results can be used in selection activities carried out in sheep-breeding.

References

1. Marmaryan G., Badalyan M., Pambukhchyan Z., Ter-Isahakyan L, The Genetic Characteristic of Edilbay Breed of Sheep Breeding in Armenia on Loci for Transferrinand Ceruloplasmin, Biological Journal of Armenia, 2014, 4, 66-70
2. Marmaryan Yu., Badalyan M., Kamalyan R., Blood Protein Polymorphism of Small Cattle Bred in Armenia, Scientific Papers Animal Science and Biotechnologies, 2017, 50 (1), p. 140-146
3. Marzanov N.S., Iolchiev B.S., Marzanova L.K., Chalaia E.A., Genetic markers at goats, XXVII Intern. Conf. on Animal Genetics, Minneapolis, USA, 2000, pp. 4.
4. Glazko V.I., Isoenzymes Genetics of Agricultural Animals, Findings of Science and Technics, Series of General Genetics, M., VNIITI., 1988, 10, 212

5. Clarke S.W., Tucker E.M., Hall S J.G., Genetic polymorphisms and their relationships with inbreeding and breed structure in rare British sheep: The Portland, Manx Soghtan and Hebrideant, Conservation Biology, 1989, 3(4), 381-388.
6. Arrieta-Aguirre I., Alvarez V., Berriatua E., Jugo B.M., Optimization of Mhc-DRBl gene typing and association with Maedi-Visna virus infection in sheep, 29th Intern. Conf. on Animal Genetics, Tokyo, Japan, 2004, pp. 58.
7. Drogemu Uer C, De Vries F., Hammann H., Ganter M. et al., PRNP polymorfisms in German sheep breeds, 29th Intern. Conf. on Animal Genetics, Tokyo, Japan, 2004, pp. 75.
8. Oin J., Lee C.Y., Whetherall J., Groth D., Identification of cosmid clones spanning the sheep MHC region, 29th Intern. Conf. on Animal Genetics, Tokyo, Japan, 2004, pp. 57.