Blood Protein Polymorphism of Small Cattle Bred in Armenia

Gayane Marmaryan*, Manvel Badalyan, Romik Kamalyan

Armenian National Agrarian University, 74 Teryan St, Yerevan 0009, Armenia

Abstract
Biochemical and genetic markers have not yet been used in selection and breeding of agricultural animals in Armenia. The objective behind the experiments was to assess the small cattle bred in Armenia- the semi-coarse wool and semi-fine wool sheep, as well as goats of different genotypes characterized by different milk productivity, polymorphic blood proteins-transferrin, ceruloplasmin, aiming to use them in breeding. Another objective was to study the genetic distance between the researched breeds and crossbreeds aiming to reveal the genetic similarity. The findings of research studies come to show that goats with polymorphic transferrin locus and a big set of genotypes are characterized by higher milk productivity; therefore it is recommended that they be used in selection as a supplementary milk productivity marker. The genetic distance between the researched goats comprised 0,60 which comes to show that semi-fine wool sheep were also bred with the help of semi-coarse wool crossbreeds. The high coefficients of genetic distance of crossbreed goats compared to both the highly milk producing imported breeds and the locals are indicative of inherited high adaptability qualities of locals and high milk producing quality of pure breeds.

Keywords: ceruloplasmin, goats, polymorphism, sheep, transferrin

1. Introduction

Sheep and goat breeding are the traditional branches of animal husbandry in Armenia. The dynamics of the total number of sheep and goats bred in Armenia for the period 2000-2016 is displayed in Figure 1, which comes to show that this branch remains a priority area in the livestock sector of the country [1]. The rich gene pool of agricultural animals formed due to both popular and scientific selection serves as a good ground for raising new breeds under today's conditions.

Increasing the milk productivity of agricultural animals remains problematic in the field of animal husbandry of the country. Given the lack of high milk-producing goats in Armenia, the research is targeted at breeding high productivity animals. For the purpose of increasing the productivity of local goats and breeding new breeds of dairy goats, high milk-producing goats - Alpine, Zaanen and Toggenburg, successfully used in breeding worldwide, have been imported to the country. In Armenia, the imported goats have been bred both through pure breeding and through crossbreeding with the local low milk-producing breeds. Crossbreed goats have been bred through selection and exceed the locals by milk productivity, while by their adaptability to natural and climatic conditions they exceed the imported pure breeds.

The research study has the following objectives: 1) to identify biochemical and genetic markers to be applied in selection as supplementary markers of milk productivity 2) to establish the degree to which the same markers can be applicable in case of various types of small ruminant 3) to monitor the gene pool of researched breeds and animals in terms of genetic distance.

The selection criteria of researched animals are as follows:

The Zaanen breed of goats is one of widespread milk producing breeds in the world. The lactation

* Corresponding author: Gayane Marmaryan, gmarmaryan@gmail.com

period of goats lasts from 10 to11 months. Per lactation period 600-700 kg of milk on average is yielded. The average content of fat in the milk is 3.8-4.5%. Zaanen goats are well acclimatized to different climatic conditions. When crossbred, they invariably transfer their useful economic properties to their generation [2].

The Alpine breed of goats is aboriginal; its milk productivity is 700-1000 kg for the lactation period of 270-350 days, where the fat content is 3.5-4.0%, while the protein content is 3%. [2, 3].

The Toggenburg breed of goats has been bred throughout many centuries through popular selection method. All of three high milk-producing imported goat breeds have been bred in the Swiss Alps. The milk productivity fluctuates within a wide range – from 400 to 1000 kg per lactation. The average content of fat in the milk is about 4% [4].

The local goats do not normally belong to any breed, they are the result of popular selection and are widely spread on the territory of Armenia. The lactation period lasts for 7-8 months, the milk productivity per lactation is 150-20 kg, the level of protein is 3.7% on average, while that of fat is 6.0%. They have high adaptability to local natural and climatic conditions.

Sheep breeding in Armenia is predominantly combined in nature - it is meat, wool, and milk oriented; however, in the recent decade it has the tendency to shift towards meat-and-milk producing ones.

The sheep of Armenian semi coarse wool breed are characterized by endurance and the ability to well accommodate to long pasture maintenance under mountain conditions. The live weight of rams (more than 1.5 years old) is 89-92 kg, while that of ewes (more than 1.5 years old) is 55-57 kg. The milking capacity of every milking sheep of Armenian semi coarse wool sheep is 115-120 for the lactation period of 150-175 days. They are characterized by good fertility, during the maternity period 100 female ewes give birth to 115-120 lambs on average [5].

The characteristics of semi fine wool sheep of corridel type in the ontogenesis testifies that the live weight of rams is 80-85kg, that of ewes - 52-56 kg. The animals are well adapted to natural and climatic conditions of foothill and mountain areas of Armenia, their milk productivity is 120-130 kg per lactation, while fertility is 115-120 lambs per 100 ewes [6].

A number of breakthrough discoveries in the field of genetics, biochemistry and molecular biology have contributed to the application of genetic markers in the selection and breeding processes of agricultural animals providing a basis for the development of molecular or marker assisted selection [7, 8]. The genetic marking makes it possible to benchmark the populations in terms of biodiversity, to monitor the gene pool of the breed, to assess and estimate the efficiency of breeding, to propose selective markers of qualitative and quantitative descriptors, to analyse processes that take place in the organisms of animals [9].

Amongst genetic markers the biochemical polymorphism of proteins is considered to be the most widespread due to a number of peculiarities, which enables not only carrying out the selection in a strictly determined direction, but also identifying the origin and the genetic similarity between various breeds. The basis for the genetic polymorphism of proteins is the multiple “allelism” of genes [10].

Transferrin, the main glycoprotein of blood which regulates the correlation between bivalent and trivalent iron ions, is characterized by bactericidal feature and inhibits the growth of viruses [11].

Ceruloplasmin is a copper containing metalloprotein characterized by oxidaze activity. It is a trivalent system consisting of migrant alleles with a fast CpA, slow CpC, and average CpB speed. It shapes one strip for homozygote forms and two strips for heterozygote forms [12].

2. Materials and methods

The subject matter of research were high milk-producing goats of Alpine, Zaanen and Toggenburg breeds imported to Armenia from the USA as well as low milk-producing local aboriginal goats and crossbreeds of the second and third generation, resulting from the crossbreeding of local and imported goats. Apart from goats, the research also covered the sheep of Armenian semi coarse breed (Aragats type) characterized predominantly by meat, wool and milk productivity as well as Armenian semi fine breed of corridel type. The researched animals were female and three years of age. Overall 51animals have been researched, of which 20 goats and 31 sheep. The goats had been bred in the “ARID”

goat breeding centre located in the city of Yeghegnadzor, Vayots Dzor region of the Republic of Armenia, as well as sheep on the farms of Kotayk and Aragatsotn regions.

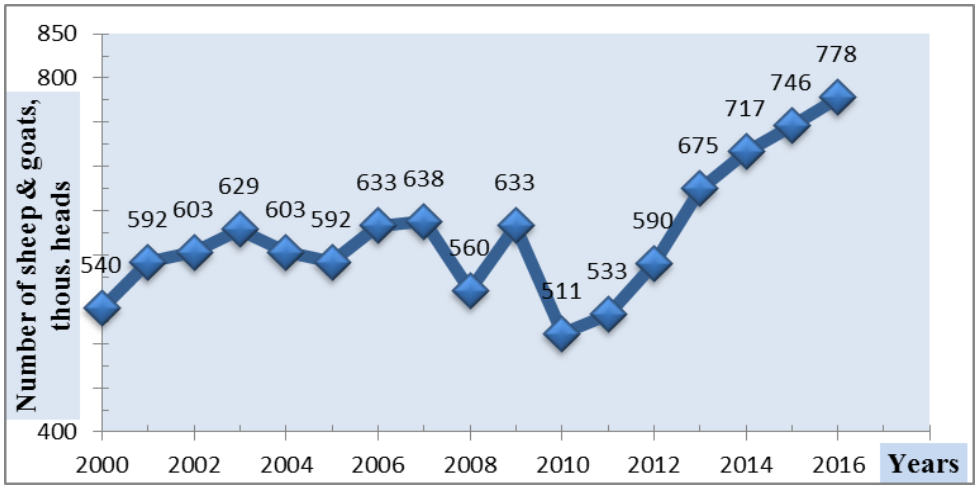

Figure1. Total number of sheep and goats in Armenia for the period 2000-2016

The blood samples for the research were taken from jugular vein, placed into the vacuum test tubes containing gel activator and were centrifuged at 6000 RPM (revolutions per minute). The research of protein polymorphism was being conducted at the "Laboratory of Agriculture, Food Safety and Technology of Environment Protection" of Armenian National Agrarian University. The set of biochemical and genetic polymorphic systems of blood researched included the study of transferrin (Tf) and ceruloplasmin (Cr) loci.

The research studies of polymorphic systems of the blood of small cattle were conducted by means of electrophoresis in PAAG (polyacrylamid gel), and in the case of transferrin and ceruloplasmin loci by means of Laemmli [13] (Biometra). For the Tf locus a 10% gel was used: in the case of the separating gel, the buffer was 1.5 M tris-HCI, pH 8.8, while in the case of the stacking gel- 0.5 M tris-HCI, pH 6.8. Tris-glycine was used as electrode buffer, pH 8.34. For the Cr locus, a 7.5% gel was prepared. Gel buffer – 0.18 M tris-HCI, pH 8.8, electrode buffer- 0.016 M tris-glycine, pH 8.7. For the purpose of gel dying the Coomassie brilliant blue R 250 was used with a further processing of electrophoregram through a 1D gel electrophoresis image analysis software Gel Analyser.

A number of formulae have been used for the purpose of analyzing the genetic systems of populations [14,15]. The frequency of genotypes and alleles was determined by the following formula: $P_i = \frac{n_i}{N}$,

where P_i is the frequency of the I allele, n_i is the number of animals with the given allele, N is the total number of researched animals. The index of the genetic distance (r) between the researched breeds was determined by Mile Lindstrem formula: $r = \frac{\sum x_1 x_2}{\sqrt{\sum x_1^2 \cdot \sum x_2^2}}$, where r is the coefficient of genetic distance, which varies between 0 -1, X_1 and X_2 – the frequency of the same alleles of the compared groups. The degree of homozygosity of the protein polymorphism was determined by Helderman fomula: $SH = \sqrt{\frac{\sum (H_1 - H)^2}{n}}$, where SH is the average indicator of homozygosity of several loci, H is the average indicator of the researched homozygosity, while n is the number of researched loci. For each locus, homozygosity was determined by an ordinary proportionality principle from the total number of researched animals.

3. Results and discussion

The marking test systems began to be applied as a tool for illustrating the genetic variation, the genetic differences amongst various populations, the evaluation of allele resources in different types and breeds, as well as for establishing a link

between a number of biochemical and physiological processes in the organisms of agricultural animals [16, 17]. The existence of genetically determined diverse shapes of various proteins helps determine the possible impact of environmental conditions and selection methods on the genetic structure of animal populations and on the gene pool of breeds while improving them. These are also applied in the selection process as a tool to specify the origins of animals, to determine the genetic proximity between various sets of organisms, and to mark the heredity of valuable animals. It is deemed that even the various breeds of the same type can differ by both the quantity of alleles, determining the polymorphism of protein systems, and by frequency of their occurrence [18].

In sheep breeding, the genetic evaluation of breeds and types with the application of various markers, including the polymorphic protein systems, acquires an ever more significance [19-22]. However, despite their obvious advantages, biochemical and genetic markers are not yet used in Armenia when researching the genome of small cattle. Given the above said, the rationale behind the series of experiments was the assessment of the small cattle bred in Armenia in terms of polymorphic blood proteins - transferrin (Tf) and ceruloplasmin (Cr), aiming to use them in breeding.

The analysis of the findings of electrophoresis of the blood serum of animals with regard to multiple protein shapes comes to prove that both transferrin (Tf) and ceruloplasmin (Cr) in the corridel type of semi fine wool sheep are polymorphic and are determined by A and B alleles, wherein the frequency of A allele was 0.25, and that of B allele was 0.75 (Table 1). What concerns the genotypes, it is to be noted, that the Tf of the corridel type of sheep is revealed through two genotypes: heterozygous AB, comprising 25% of all researched animals, and homozygous BB - 75%, meaning that A-allele is determined by homozygous genotype, while B-allele - by heterozygous genotype. The degree of Tf heterozygosity comprises 75%.

With regard to Cr, it should be noted that it is polymorphic, too and is comprised of A and B alleles, the frequency (Pi) of which is 0.31 and 0,69 respectively. In the corridel type of sheep, Cr as opposed to Tf is represented by two heterozygous genotypes (AB 31.3% and BC 50%) and one homozygous genotype (BB 18.7%). The degree of Cr homozygosity comprises 18.7%. Thus, the degree of Tf and Cr homozygosity of the corridel sheep type comprises 66%.

Table 1. Polymorphic system of the blood of semi coarse wool and semi fine wool sheep of corridel type in regard to Tf and Cr

Locuses	**Alleles**	**Semi fine wool / n =16 /**		**Semi coarse wool / n =15 /**	
		Genotypes %	Allele frequency Pi	Genotypes %	**Allele frequency Pi**
Tf	A	AB 25	0.25	AA 33.3	**0.73**
	B	BB 75	0.75	AB 26.6	**0.27**
	C	-	-	AC 13.4	-
				BB 20.0	-
				BC 6.7	
Cr	A	AB 31.3	0.31	AA 13.3	**0.13**
	B	BB 18.7	0.69	AB 73.4	**0.87**
		BC 50.0	-	BB 13.3	-
Homozygosity, %		66	-		**32.1**

Table 1 also displays Tf and Cr data on Armenian semi coarse wool breed, making it obvious that in this case, too, Tf is polymorphous and is determined by A and B alleles, wherein the frequency of A allele was 0.73, and that of B – 0.27, the difference being that in sheep of Armenian semi coarse wool breed the A and B alleles of Tf are revealed through 5 genotypes- AA-33.3%, AB-26.6%, AC-13.4%, BB-20% and BC-6.7%, wherein the highest frequency is being

registered in the AA genotype, the lowest - in the BC. The Tf homozygosity in the sheep of semi coarse wool breed comprised 53.3%.

The locus of Cr sheep with semi coarse wool, as in the case of semi fine wool, is polymorphous and consists of A and B alleles, where the frequency of the A allele was 0.13, while that of B – 0.87. The data displayed in the Table come to prove that in terms of Cr genotype the sheep of Aragats type do not yield to semi fine wool: AA-13.3%, AB-73.4%, BB-13.3%, the only difference being that A allele in corridels is revealed through AB genotypes, while in semi coarse wool – through AA and AB genotypes. To conclude, Cr in both groups of researched sheep is represented by three genotypes, which, apparently, proves that both semi coarse wool and semi fine wool sheep are characterized by meat, wool, and milk productivity. The Cr heterozygosity of the sheep of this breed comprises 26.6%. What concerns the degree of homozygosity of Tf and Cr indices, in semi coarse wool sheep it made up 32.1%, yielding to semi fine wool breed by approximately 50%.

The findings on Tf and Cr multiformity of goats are displayed in Table 2. The research results were studied and analysed based on the genotype of animals: highly milk producing imported breeds, locals and crossbreeds. It is worth noticing that the blood Tf of highly milk producing imported goat breeds is polymorphous and consists of A, B, and C alleles, wherein the frequency of the A allele is quite high – 0.78, while the B and C alleles have equal, however, slow frequency rate – 0.11. Naturally enough, the allele which displays a high frequency rate, forms several genotypes: thus, the A allele in highly milk producing imported goats is expressed through AA, AB, and AC genotypes, with 22.2%, 33.2% and 22.3% respectively. The B allele of Tf forms one BB genotype, comprising 11.1%. As to the C allele, it is expressed through one CC genotype too, the percentage of which is analogous with the B allele and makes up 11.1. According to our data, homozygous Tf genotypes of highly milk producing goats makes up 44.4% of total genotypes, i.e. the degree of Tf homozygosity is 44.4%.

The biochemical research studies on multiformity of the blood Tf of local breeds of goats have shown that Tf is monomorphic, consists of one A allele, the frequency of which equals to 1.0. With regard to generated genotypes, it should be noted that there are three of them- AA 25%, AB 50% and AC 25%. The degree of homozygosity of Tf locus in the local goat breed is 25%, yielding to highly milk producing imported breeds by 19.4%.

A slightly different picture is observed in crossbreed goats. The data in the Table shows that like in the case of highly milk producing goats, in this case, too, the Tf is polymorphic, consists of A and B alleles, the frequency of the A allele, as in the case of imported breeds, is very high and makes up 0.71, while that of B, makes up 0.29. The specified alleles are being expressed by four genotypes - AA 29%, AB 14%, AC 28%, BB 29%. The B allele in crossbreeds, as in highly milk producing imported ones is expressed by one, however, a homozygous genotype. The degree of homozygosity of crossbreeds in Tf comprises 58%, which exceeds the locals by 33%, and the highly milk producing breeds of goats by 13.6%. This difference may be due to a different genealogy of the breeds, and possibly, also due to the impact of various natural and climatic conditions on the formation of their heredity.

Quite an interesting picture is being observed with regard to Cr. The data in the Table show that in all groups of researched goats it is monomorphic and consists exclusively of the A allele, the frequency of which equals to 1, is expressed by AB genotype, which equals to 100% in all cases. The Cr homozygosity comprises 0%; consequently, in all groups of researched goats the degree of homozygosity is expressed solely by the percentage of Tf homozygosity.

When comparing the indices related to the frequency of the alleles and genotypes of animals belonging to breeds of the same type, there arises a necessity to determine the genetic distance between them.

In our research studies, we have revealed that the genetic distance between the semi fine wool sheep of corridel type and the Armenian semi coarse wool sheep equalled to 0.60, which comes to prove that the semi fine wool sheep of corridel type were also bred by using semi coarse wool breeds. The crossbreeds have shown a rather high coefficient of genetic distance both between the locals, equalling to 0.96, and to pure breeds – 0.88, which comes to prove that the crossbreeds have inherited high adaptability qualities of the locals and high milk producing quality of pure breeds.

Table 2. Tf and Cf polymorphic system of the blood of different goat breeds and crossbreeds

Locuses	Alleles	Imported breed		Local breed		Crossbreeds F_2, F_3 generation	
		Genotypes %	Allele frequency Pi	Genotypes %	Allele frequency Pi	Genotypes %	**Allele frequency Pi**
Tf	A	AA 22.2	0.78	AA 25	1.0	AA 29	0.71
	B	AB 33.3	0.11	AB 50	-	AB 19	0.29
	C	AC 22.3 BB 11.1 CC 11.1	0.11	AC 25	-	AC 18 BB 29	
Cr	A	AB 100	1.0	AB 100	1.0	AB 100	1.0
Level of Tf locus homozygosity %			44.4		25.0		58.0

K.S. Rodriguez [23] notes that polymorphic systems of blood and milk proteins are inherited co-dominantly, do not change in the course of the whole life of the animal, and, therefore, can serve as suitable genetic model when resolving selection related theoretical and practical issues.

As a result of research studies, Sadikulov and Kim [24] note that the sheep of breed with TFAC-CpBB, TFCC-CpAB genotype had a high carcass weight and a first category meat compared to animals with TFCC- CpBB, TFAC-CpAB genotype.

According to data by A. Lazowski, ewes with TFBC genotype differ by polycarpic feature as well as high preservation of youngsters [25].

4. Conclusions

1.We have displayed the productivity related differences between polymorphic forms of transferrin – the ferrum transferring protein - in different groups of goats and sheep bred in Armenia. It has been revealed that goats with polymorphic Tf and a big set of genotypes have higher milk productivity; therefore, it is reasonable to use them in selection as supplementary selection criteria of milk productivity in the case of goats.

2.It has been revealed that the genetic difference between the corridel type of sheep and the semi coarse wool breed comprises 0.60. This comes to prove that semi fine wool sheep have been bred with the use of semi coarse wool crossbreeds. The high coefficients of genetic distance of crossbreed goats compared to both imported and local ones, as well as the changes in metabolism indices, come to demonstrate inherited high adaptability qualities of the locals and high milk producing quality of pure breeds.

Proposals: Given that highly milk producing breeds of goats bred in Armenia, with polymorphic Tf locus and a big set of genotypes are characterized by higher milk productivity, it is recommended that Tf be used as a supplementary milk productivity selection marker.

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