

GENETIC POLYMORPHISM OF SERUM ALBUMIN IN THE CARPATHIAN BREED IN ECONOGENIC INTERRELATION WITH OTHER GOAT BREEDS

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Abstract

This paper has proposed to reveal the albumin polymorphism in goats belonging to the Carpathian breed. Identification of albumin phenotypes was performed from blood serum of animals by starch gel electrophoresis method. Depending on the migration speed of albumin fractions, three phenotypes were found: homotype AlbFF (with fast electrophoretic migration), homotype AlbSS (with slow electrophoretic migration) and heterotype AlbFS (with intermediate electrophoretic migration). Emergence of albumin phenotypes is due to the two co-dominant autosomal alleles, Alb^F și Alb^S, whose distribution is unequal within the population, the allele Alb^F being very common (83.66%) compared to allele Alb^S (16.34%). Unequal spread of these alleles causes a differentiated distribution of albumin genotypes: homozygotes Alb^SAlb^S are the most common (70.24%), heterozygotes Alb^FAlb^S are enough well represented (26.83%), and homozygotes Alb^FAlb^F register a very low frequency (2.93%). As a result, the summed homozygosity (Alb^FAlb^F + Alb^SAlb^S) is three times more frequent (73.17%) than heterozygosity Alb^FAlb^S (26.83%). The Carpathian breed is in Hardy-Weinberg genetic equilibrium at the locus Alb, both at the level of individual albumin genotypes, as well as regarding the general status of albumin zigosity. From the econogenic point of view, the Carpathian breed is similar to several breeds of goats of the Central and Eastern Europe.

Key words: albumin, genetic polymorphism, goat

INTRODUCTION

Broadly, the serum albumins constitute a very heterogeneous group of protein fractions which are represented electrophoretically by the most advanced migration front.

By the distance at which the electrophoretic bands are positioned to the starting line of the albuminic electrophoregrams, the serum albumins include three major groups: pre-albumins, albumins and post-albumins. Each group has more fractions with polymorph character, each of them presenting certain specific biophysical, biochemical and metabolic peculiarities [12].

From the biochemical point of view, the serum albumins are the most studied proteins both humans and animals (especially the farm ones) because the albumin is the most important and complex protein of the blood serum, having the most important physiological valences in the metabolic economy of the animal body, as maintenance of colloidal state of the cytoplasm,

compensation of hydrostatic pressure exercised within the capillaries, adjustment of blood osmotic pressure, transport of metal ions, vitamins, hormones, fatty acids etc. [12, 13].

If the proper serum albumins are well known in quantitative terms, about their polymorphism in the caprine species it can be said that the study is less thorough. The first data on albumin polymorphism appeared for the first time in 1964, in the Norwegian breeds Spael-South, Spael-North and Dala, being offered by Efremov and Braend, in which three genetic variants were reported [2].

Because of this issue, the need to study the genetic structure at the Alb locus in the Carpathian goats seemed not unimportant in order to identify the albumin polymorphisms in this breed and to use this biochemical polymorphism for optimizing the improvement programs and breeding technologies of this goat breed.

MATERIAL AND METHODS

The biological material necessary to achieve the proposed experiment consisted of a random population of goats belonging to the Carpathian breed exploited at the Research and Development Station for Sheep and Goat Breeding, Popauti-Botosani.

The analyzed biological fluid was blood sampled, without anticoagulant, by jugular venipuncture of the animals.

Determination of serum albumin types

The serum albumin genetic variants in goats were determined by horizontal electrophoresis method, similar to the method of determining the transferrin, distinguished from that by the reaction character of Tris-citrate buffer, moved towards the neutral zone (pH=7.6), and especially by replacing the lithium hydroxide from lithium-borate electrolyte with sodium hydroxide, but which does not alters essentially the reaction character (pH=8.7).

Preparing the solutions.

- a) *Tris-citrate buffer*, stabilized at pH=7,6:
 - tris(hidroxiomethyl)aminomethane ...1,60 g
 - citric acid0,91 g
 - distilled waterad 1000 ml.
- b) *Sodium-borate buffer (electrolyte)*, in discontinuous system, stabilized at pH=8.7:
 - boric acid18.55 g
 - sodium hydroxide4.00 g
 - distilled waterad 1000 ml.
- c) *Gel buffer*, stabilized at pH=7,8:
 - tris-citrate buffer.....9 parts
 - lithium-borate electrolyte1 part.
- d) *Blood serum*. After retraction of coagulum from the blood samples, the serum was centrifuged at 3000 r/min. The supernatant serum thus obtained was stored at -30°C being susceptible for electrophoresis analysis.

Preparing the electrophoretic substrate.

The starch gel was prepared of 100 ml gel buffer to which were added 12.5 g hydrolyzed starch. This mixture was boiled until liquefaction and all air bubbles were removed using a vacuum pump. Then, the liquefied mixture was moulded on a rectangular glass plate (260mm x 160mm). After the gel has acquired consistency, incisions were made in it, representing the places where the Whatman inserts, embedded in the sera examined, were placed.

Electrophoretic migrating. The gel plates were placed in a perfectly horizontal position in the electrophoresis apparatus. The Whatman insertions loaded with serum samples were inserted at 4 cm from the starting line. The electrophoresis lasted 6 hours until the electrophoretic bands migrated to six cm from the starting line. The power parameters for electrophoretic migrating were 50 mA (intensity) and 400 V (tension).

Development of albumin electrophoregrams

The colouring of the starch gels was made in an alcoholic-acid solution of amidoschwartz 10B, 2%, for 15 min.:

- amidoschwartz 10B20 g
- methylic alcohol 455 ml
- glacial acetic acid 90 ml
- distilled water 455 ml

The discolouring of the starch gels was made in 2-3 successive baths of decolourizing solution, for more hours, until the gel got clarity:

- methylic alcohol 2275 ml
- glacial acetic acid 250 ml
- distilled water 2275 ml

The identification of albumin phenotypes was achieved depending on the migrating speed of the electrophoretic bands. The albumin which presented the fastest migrating speed in the substrate electrophoretic was named albumin of „fast type” and the one whose band is closest to the application points of serum samples was designed albumin of „slow type”. Presence of both types of albumin in the same animal determines appearance of bands with intermediate moving in the electrophoretic field, representing albumin of „fast/slow type”.

The experimental results were analyzed statistically. On the basis of albumin phenotype incidences, there were calculated the frequencies of albumin genes and genotypes, as well as the frequencies of homozygotes and heterozygotes from the Alb locus level. In relation to the observed frequencies of albumin genotypes, their estimated frequencies were calculated. The comparison between observed and expected frequencies was done by the Hi square test (χ^2), establishing the conformity of the Carpathian goat breed to the Hardy-Weinberg law of genetic equilibrium.

RESULTS AND DISCUSSIONS

An examination of serum protein polymorphisms in Carpathian goats led to observing the differences in electrophoretic patterns of the protein migrating in the albumin zone. Analysis of albumin electroforegrams reveals the existence of multiple molecular forms of this protein that confer to it the characterial discontinuity.

These genetic variants, reflected in the electrophoretic plan, are, in fact, albumin phenotypes, whose encoding is Alb^F for the "fast type" (with fast migration), Alb^S for the "slow type" (with slow migration) and Alb^{FS} for the "fast/slow type" (with intermediate

migration). The homotypes - Alb^{FF} and Alb^{SS} - are marked by two denser bands in the electrophoretic field, the slower (cathode) band being the most intensely coloured. The slowest one of the two bands of phenotype Alb^F migrates at a rate that is synchronous with the fastest (anodal) one of the two bands of phenotype Alb^S. The heterotype Alb^{FS}, which displays three bands, appears as a compound of phenotypes Alb^F and Alb^S in which the slow and intermediate bands are more intensely coloured than the fast band (fig. 1). Some authors identify the intermediate phenotype by four bands, the more colourful spots alternating with blurred spots.

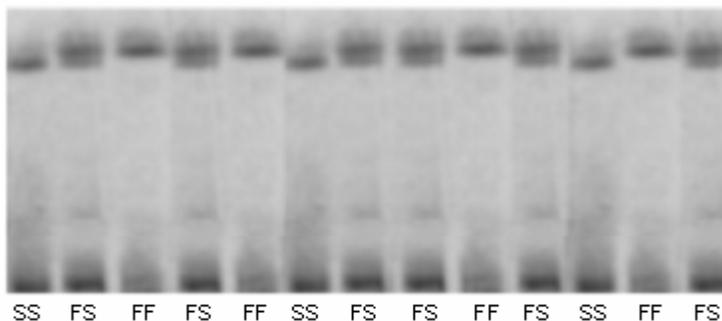


Fig. 1. Electrophoretic patterns of serum albumin in the Carpathian goats

These observations suggest that the three albumin phenotypes are under the control of a pair of co-dominant autosomal alleles, designated Alb^F and Alb^S. These two alleles determine expression of three albumin genotypes: two homozygous, Alb^FAlb^F and Alb^SAlb^S, and one heterozygous, Alb^FAlb^S.

In the Carpathian breed, allele Alb^S registers a high frequency (83.66%), and its co-dominant Alb^F has a relatively low incidence (16.34%) (fig. 2). As a result of these allelic distributions, the albumin genotypic table is dominated by the homozygotes for allele Alb^S (70.24%), while those for allele Alb^F have a very low spreading (2.93%). The heterozygotes Alb^FAlb^S have a moderate but considerable representation, occupying a quarter of the albumin table (26.83%) (fig. 3). For this reason, the total homozygosity (Alb^FAlb^F + Alb^SAlb^S) (73.17%) is more frequent than heterozygosity (Alb^FAlb^S) (26.83%), the ratio between the two status of the albumin zygosity being 3 / 1 (fig. 4).

From a statistical viewpoint, the genotypic frequencies found do not deviate

from the Hardy-Weinberg law expectations, leading to extremely low and completely insignificant values of the χ^2 test, proving that the breed is in genetic equilibrium, both as individual distribution of the albumin genotypes, and as a general representation regarding the proportions of homozygosity and heterozygosity (fig. 3, 4).

The Carpathian breed is part of the goat group in which all three albumin genotypes are expressed: Alb^FAlb^F, Alb^FAlb^S and Alb^SAlb^S. In breeds of this group, the albumin table is dominated by the homozygotes for allele Alb^S (between 60% and 95%); the heterozygotes Alb^FAlb^S have a relatively low spreading (about 15%, exceptionally 20%) or very limited (2%-5%), and the homozygotes of Alb^FAlb^F type have a relatively low frequency (10%-15%) or their presence is sporadic (0.5%-2%) [1, 9, 11, 13, 14]. However, the Carpathian breed, compared to other breeds, represents a slightly atypical case. In this breed too, homozygotes of Alb^SAlb^S type are predominant (70%), but heterozygotes for both alleles have a substantial

representation (almost 28%), and the presence of the homozygotes for allele Alb^F is extremely small (almost 3%). Among all breeds, the Carpathian breed would have some genetic

similarities with the Saanen and Alpine breeds of the Central and Eastern Europe, regarding the distributions of albumin genotypes.

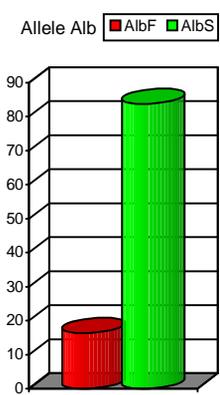
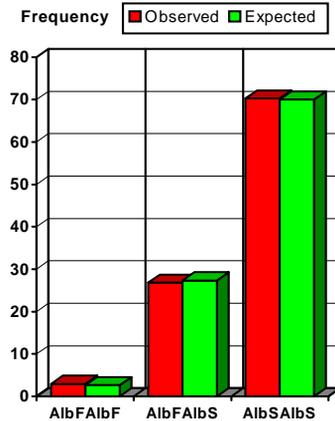
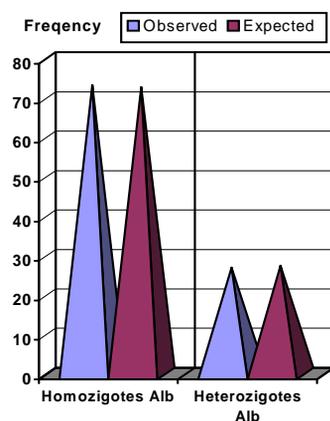


Fig. 2. Allelic structure at the locus Alb in the Carpathian goats



$\chi^2 = 0,0357$; G.L.=2; $p > 5\%$
Fig. 3. Genotypic structure at the locus Alb in the Carpathian goats



$\chi^2 = 0,0131$; G.L.=1; $p > 5\%$
Fig. 4. Zygosity status at the locus Alb in the Carpathian goats

This group of goats, with a ubiquitous spreading, differs, in terms of the genetic structure at the locus Alb, of the group of local (native) goats, in which the albumin polymorphism is restricted, being expressed only two genotypes. There are two instances of this kind. Firstly, in some local breeds from Italy (Luciano breed) [10] and Spain (Murciana breed) [1], the heterozygotes Alb^FAlb^S are preponderant, homozygotes Alb^SAlb^S register a low frequency, and the homozygotes of phenotype Alb^F missing from the albuminic table. In the second case, other ecotypes of Saanen goats from Italy and Hungary [3, 10], local breeds from Japan [13] and Serrana Andaluza breed from Spain [1] have more homozygotes for allele Alb^F and less heterozygotes Alb^FAlb^S, and the albumin homozygotes with phenotype Alb^S are not present. In a local breed of Korea, the homozygotes with fast electrophoretic migration (Alb^SAlb^S) missing, and the homozygotes for allele Alb^F and heterozygotes Alb^FAlb^S have comparable frequencies, with some surplus for the homozygous individuals (53,2%/46,8%) [4].

In the context of a more accentuated isolation, some local breeds from China [5], South Africa [7], Japan [6] or even Europe

(some ecotypes of Saanen from Switzerland or France) [8, 13] tend to monomorphism to the locus Alb, the only genotype expressed being the homozygous one for allele Alb^S. It is possible that in this case to be able to talk about a *transmutation* phenomenon of the polymorphic albumin system in a monomorphic system.

Thus, as in sheep, the hypothesis has been postulated that, in goats too, the polymorph system can be transmuted into monomorph system during evolution in the Alb locus. In this context, the polymorphism can be transitive, turning toward monomorphism as a result of elimination of some alleles under the influence of environmental conditions, combined with the pressure of technological and selection factors. In this way, the breed becomes monotypic, after the preponderant allele during polymorphism is the only allele present during monomorphism (usually, the allele Alb^S).

The differentiation of goat breeds in the world must be seen in much broader sense, in econogenic context, in which different factors can individualize the immunogenetic profile of each breed of goats, such would be:

- conditions of geographic environmental and microclimate in which the animals live;

- selection systems applied for improving the breeds;
- breeding technologies used for exploitation of animals;
- socio-human factors relating to traditional and cultural aspects of the local people communities;
- involvement degree of the scientific phenomenon in the livestock practice;
- reproductive isolation caused by geographical, selection and technological factors;
- peculiarities of production metabolism of the animals (specialization for certain morpho-production types: meat, milk, hides, wool, such would be the mohair and cashmere wool).

All these factors are real prerequisites to enhance the biodiversity of these species, essential condition for its improvement, from the viewpoint of the production, reproduction and health parameters

CONCLUSIONS

1. In the goats of Carpathian breed, the expression of the albumin polymorphism is of middle level, having a binary structure.

2. Depending on the migration speed, three albumin phenotypes have been identified in the electrophoretic field: Alb^F (fast homotype), Alb^{FS} (intermediate heterotype) and Alb^S (slow homotype).

3. The albumin phenotypes are the expression of a pair of co-dominant autosomal alleles, designated Alb^F and Alb^S, whose spreading is unequal in the population; allele Alb^S registers a high frequency, and its co-dominant Alb^F has a relatively low incidence.

4. The two alleles control the three genotypes albumin: two homozygous (Alb^FAlb^F and Alb^SAlb^S) and one heterozygous (Alb^FAlb^S).

5. The albumin genotypic table is dominated by homozygotes for allele Alb^S, while those for allele Alb^F have a very low spreading; the heterozygotes Alb^FAlb^S have an important representation. Therefore, the summed homozygosity is more common than the heterozygosity.

6. The differentiated distributions of albumin genotypes did not affect the Hardy-Weinberg genetic equilibrium at the Alb locus of the Carpathian breed.

7. In econogenic terms, the Carpathian breed may relate to several breeds of goats of the Central and Eastern Europe.

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