

ASSOCIATION OF THE HAEMOGLOBIN GENOTYPES WITH QUALITATIVE PRODUCTION TRAITS OF LAMBS BELONGING TO THE BOTOSANI KARAKUL BREED

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The production specificity of Karakul sheep is lamb pelt that presents an obvious aesthetic design very required in the fur clothing industry. The overall appearance of this production parameter is given by some morphological, physical and histochemical properties of pilose lamb coating estimated in the first three days after birth. The paper makes an associative analysis of the haemoglobin genotypes with qualitative features of lamb pelts in the Botosani Karakul breed sheep. The study took into account the appropriateness of using the haemoglobin types as genetic markers in the improvement works of this production trait. The associative analysis revealed the morphological and production superiority of the Hb^AHb^B heterozygotes compared to homozygotes for Hb^B allele (the Hb^AHb^A homozygous genotype missing in this breed) in terms of overall lambskin quality. The χ^2 test values show that the two haemoglobin genotypes (Hb^AHb^B and Hb^BHb^B) differ very significantly concerning this production parameter. The configuration of R x C contingency table reveals an interrelation of the qualitative features thus contributing to the overall aspect of the lamb pelts; the correlations of these features are more intense and more significant in Hb homozygotes than in Hb heterozygotes. It is necessary to increase the heterozygosity at Hb locus in the Botosani Karakul breed to improve the lambskin quality.

Key words: Haemoglobin polymorphism, lamb pelt, lambskin, Karakul sheep.

INTRODUCTION

Sheep of Karakul type has a number of biological, morphological and production features. One of them is of prime importance and refers to the beautiful fur of the newborn lamb which has a unique appearance. From a commercial perspective, the lamb pelt of Karakul type is considered to be part of the luxury fur category, being assessed at the same fashion level with the noblest natural furskins (Sobol, mink, fox). This is explained by superior aesthetic and ornamental qualities of curling, excellent silky quality of hair, quite special lustre of pilose coating, richness of colours and shade diversity of these colours generated by the melanocytes that impregnates the hair fibre content, perfect thermal qualities as well as a

sustainable resistance concerning the use of lamb pelt garments and handicraft items. To these features it can be added the “ecological fur” particularity attributed the lamb pelts of Karakul type^{2, 13, 14, 17}. All this complex of traits makes of Karakul sheep perhaps the noblest sheep breed and the Karakul lambs, by curling drawing and chromatic variety, are the most beautiful in the world having an elegant and graceful appearance.

Despite competition which makes them the imitative industry of synthetic furs, the Karakul lamb pelts continue to be required, as raw materials both for light ready-made clothe industry and for the artisanal one, due to the elegance, utility and sustainability of garment made from them such as: coats, blazers, jackets, hats, caps, hats, collars, gaskets, handbags, swimwear, gloves, ties; the lamb pelts come either from classic Karakul – *Persian lamb* – or from flattened

Karakul – *Persian broadtail lamb* – or from breitschwanz (foetal) Karakul – *Broadtail lamb*^{2, 7, 8, 12, 17}.

A certain influence on the qualitative aspect of lamb pelts exerts some external (non-genetic) factors that refer to breeding technologies or ambient conditions such as: feeding level of pregnancy phase, gestation period, mother sheep prolificacy and environmental factors (climate, soil, vegetation)^{1, 6, 16, 17}. But, in the greatest extent, these qualitative traits with high heritability of lamb pelts have a strong genetic determinism: genetic combinative compatibility of parents, efficient mating variants, the improvement degree of the flock, existence of improver rams by after progeny qualities, physiological health etc.^{1, 3, 5, 15, 16, 19}. The contribution of genetic factors to the externalization of qualitative features of Karakul lamb pelts is inextricably linked to the selection criteria applied by sheep improver in relation to its preferences to get a certain type of lamb pelt^{4, 13, 14, 17}. Also the biochemical-genetic systems fall into the category of genetic factors representing protein and mineral biostructures (haemoglobin, transferrin, albumin, blood group factors, erythrocyte potassium

etc.) which, due to their polymorphism, can be used as genetic makers to identify valuable economic traits^{10, 11, 18}.

In this respect, our concerns fall on this line too, concerning the association of haemoglobin genotypes with qualitative features of lamb pelts belonging to the Botosani Karakul breed as an important step of integrating the biochemical polymorphism in a complex system of improvement technologies of this sheep breed.

MATERIALS AND METHODS

The experiment was carried out on a population composed of 338 lambs of Botosani Karakul breed within the elite farm of the *Research and Development Station for Sheep and Goat Breeding Popauti-Botosani*.

The *Botosani Karakul sheep breed* is a biological creation of the researcher team from this resort, these sheep being specialized in the lamb pelt production and bred in several zootechnical lines and six colour varieties (black, greyish, brown, grey, pink and white) (Fig. 1).



Fig. 1. Lambs of the Botosani Karakul breed.

The biochemical-genetic typification of Karakul lambs at the determinant locus of haemoglobin was performed by the horizontal electrophoresis method. The electrophoretic substrate was represented by starch gel. The composition of the electrolyte consisted of a buffer solution comprising Tris (hydroxymethyl) aminomethane (9 g), boric acid (1 g) and Na₂ EDTA (0.75 g) in

100 ml of distilled water. The electrolyte pH was adjusted to 8.9. The gel buffer solution had the same composition as the electrolyte but much more diluted (3.5 times). The starch concentration in gel was 12.5%. The Whatman inserts soaked in haemoglobin solutions were introduced in the incisions from the starch gel. The gels with haemoglobin samples for phenotypic analysis were

subjected to electrophoretic migration with the help of a power of 50 mA and 400 V. Migrating lasted until the electrophoretic bands were 3–4 cm away from the starting line. Colouring of starch gels was done with amidoschwartz 10B dissolved in methanol and glacial acetic acid for 15 minutes, the concentration of the colorant solution being 1%. Then the gels were subjected to discolouration in a solution of methanol (3 volumes), glacial acetic acid (1 volume) and distilled water (3 volumes).

The qualitative assessment of lamb pelts (Fig. 2) was carried out by a complex multi-criteria estimation method. Of all the qualitative features of lamb pelts, there were taken into account the most important four of them which, in fact, define the economic value of lamb pelts (curl type, curl size, hair quality and hair fibre lustre). In their turn, these features are configured by several characteristics as follows:

- the curl type (shape), with the characteristics: cylindrical tube, tube+grain, grain, flat tube, heterogeneous (miscellaneous, irregular and deformed shapes);
- the curl size, with the characteristics: middle, middle-small, small, big;
- the quality of the hair fibre, with the characteristics: silky, normal, rough, soft;
- the lustre of the hair fibre, with the characteristics: intense, good, satisfactory, weak-metallic-mat.



Fig. 2. Botosani Karakul lamb pelts.

The parameter that was the basis of statistical processing of the experimental data was the frequency by which the incidences of characteristics of main qualitative features of lamb pelts were calculated according to haemoglobin genotypes of lambs. Order to see the qualitative differences of lamb pelts among the haemoglobin genotypes the H_i^2 (χ^2) test was used by the comparing method of empirical distributions. In

order to quantify the interrelationships among the qualitative characteristics that define the morphological assembly of lamb pelts a variant of the χ^2 test was used, called the $R \times C$ contingency table, specific test for the qualitative correlations.

RESULTS AND DISCUSSION

Haemoglobin inheritance way in sheep is co-dominant, the genotype being identified with the phenotype. In terms of *genetic structure at the Hb locus*, the ovine species is characterized by the existence of three haemoglobin genotypes, which are represented in the electrophoretic field by several migration bands, their position being a function of mass and electric charge of the haemoglobin molecule. Due to the high specialization degree of the Botosani Karakul sheep, only two haemoglobin genotypes have met in this breed: the $Hb^A Hb^B$ heterozygous genotype represents the phenotypic expression of intermediate haemoglobin ($HbAB$) identified by two electrophoretic strips with different colour intensities, the anode strip being lighter than the cathode one; the $Hb^B Hb^B$ homozygous genotype is identified electrophoretically by a darker band toward the cathode and represents the slow haemoglobin phenotype (HbB) (Fig. 3). The fast haemoglobin type (HbA) corresponding to the $Hb^A Hb^A$ homozygous genotype is missing in the Botosani Karakul breed, but it occurs in other sheep breeds being recognized by a darker band towards the anode. In the Botosani Karakul breed the haemoglobin panel is dominated by the $Hb^B Hb^B$ homozygous genotype (around 87%); the $Hb^A Hb^B$ heterozygotes recorded significantly lower frequencies (about 13%)¹⁰.

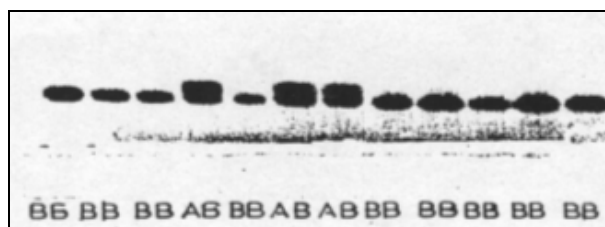


Fig. 3. Haemoglobin electrophoregram in the Botosani Karakul sheep.

Totality of qualitative features of pilose coating of a Karakul lamb constitutes the *lamb pelt curling* which, as a whole, defines the zooeconomic value of the lamb. Basically, the curling

represents the resultant of physical, mechanical, histochemical and morphological peculiarities of hair fibres structured into curls. Qualitative assessment of lambskins revealed that in the Botosani Karakul lamb population the superior characteristics of lamb pelt features predominate, while the worthless ones recorded lower or very low incidences.

The *curl type* or *shape* is the most important qualitative feature of a lamb pelt. The curl configuration is determined by the arrangement of hair fibres in bundles, their curving direction in a certain sense or curving angle, fibre density, closing degree of curl. In the survey population the curls in “cylindrical tube” shape (Fig. 4) predominate, having middle size and high closure degree and showing a good uniformity and classical drawing of Asian type (34.69%); the incidence of the other classical shape, the “grain”

one (is like a bean) (Fig. 5), is half of the previous type (16.87%). The combined curl type (“tube + grain”) (Fig. 6) recorded an average frequency between the two classic curl shapes (20.71%). Currently, it is necessary the selection to be orientated toward the lamb pelts with flattened curling generated by incompletely closed tubes (“flat tubes”) (Fig. 7), too, with short, silky and flexible hair, intense lustre and a closure degree up to a maximum of 3/4. The “breitschwants” type is a variant of the foetal flat tube. The presence of lambs with such flattened curls is considerable (18.93%) (Fig. 8). A relatively small group of lambs (6.81%) have lambskins with low economic value because of the presence of miscellaneous curls with irregular, undefined and deformed shapes (like rings, corkscrew, snails) with mat aspect and opened fibre bundles, causing an uneven curling of “heterogeneous” type (Fig. 9).



Fig. 4. Lamb pelt with curls in “cylindrical tube” shape.



Fig. 5. Lamb pelt with curls in “grain” shape.



Fig. 6. Lamb pelt with curls in “tube+grain” shape.

Fig. 7. Lamb pelt with curls in “flat tube” shape.





Fig. 8. Lamb pelt of breitschwanz type.



Fig. 9. Lamb pelt with "heterogeneous" shape curls.

The curl as pilose coating unit of Karakul lambs possesses certain dimensions (width, height, length) defining the *curl size*. The relationships among these dimensions, to which also the hair fibre density compete anymore, determine the characteristics of this quality trait. The "middle" and "middle-small" curl sizes are characteristics which give high zoo-economic value to lambskins and are found most often in the population lambs: 48.23%, respectively 34.02%. The "small" curls are in a moderate proportion (14–20%) and the "big" curls have a low spreading (3.55%). The last two curl sizes must be eliminated from population by selection because worse the lambskin quality.

The *hair quality* is a feature of lamb pelt curling determined by the thickness, density, length, uniformity and elasticity of hair fibres. But this quality is determined in the highest degree by the histological and chemical structure of the hair, especially by the internal structure of the cuticular layer; to a certain extent this quality can be influenced by structural particularities of the medullar zone of hair strand. These particularities give to the hair fibre a more silky aspect or conversely a rougher and more friable character. The peculiarities of histochemical and morphological structure of the hair fibres gives to the Karakul lamb pelts a special "silky" aspect to most individuals (76.63%), a characteristics which should be predominantly in the improver attention. In approximately 20% of lambs the hairs present "normal" quality, trait accepted in the selection process. In low proportions, the pilose coating of lambs has undesirable qualities in the selection, of "rough" (1.48%) or "soft" (2.37%) type.

The *pilose coating lustre* in the Karakul lambs represents the property of hair fibres and curls to reflect more or less the light rays. This property of fibres is conditioned by the density of fibres and

curls, curving angle of fibres, but especially by the cuticular layer compactness; this last particularity is provided by the shape, size, contour and the adherence degree of cuticular cells (scales), their disposition uniformity and smoothness degree of this cellular surface. These properties of cellular morphology provide to the Karakul lamb pelts, preponderantly, a "good" lustre (77.51%), although the most important characteristics pursued in selection is the "intense" lustre (16.57%). The "intense" lustre is very difficult to obtain and requires the application of some very laborious improvement procedures. The inferior lustre of the fibre rarely occurs in population such as the "satisfactory" one (5.32%), or accidentally such as the "weak-metallic-mat" one (0.60%).

Comparing the two lamb subpopulations by their belonging to haemoglobin genotypes, obvious differences were observed between them in terms of lambskins features.

a) Association of haemoglobin genotypes with curl shape (type) (Fig. 10)

The "cylindrical tube" shape was long time considered the most valuable curl shape and selection was carried out in this direction. The frequency of this curl type is clearly superior in $Hb^A Hb^B$ heterozygotes compared to that found in $Hb^B Hb^B$ homozygotes. The incidences of "tube + bob" and "flattened" types are almost similar in the two subpopulations, with a slight advantage for Hb homozygotes. At the same time, lambs with "grain" shape curls are over two times less spread within the Hb heterozygotes than within the Hb homozygotes. Generally, the valuable curl types have a wider spread in the $Hb^A Hb^B$ heterozygous lambs than in the $Hb^B Hb^B$ homozygous lambs. Conversely, the curls with low economic value forming the "heterogeneous" type are associated

with almost 1.7 times more with the homozygous genotype for the Hb^B allele compared to the heterozygous genotype at the same locus.

b) Association of haemoglobin genotypes with curl size (Fig. 11)

The ideal size of the curling, the “middle” one, is more common in heterozygotes Hb than in Hb homozygotes, and the “small-middle” size has almost a similar distribution in both haemoglobin genotypes, slightly higher in homozygotes. The valuable sizes of the curling (“small” and “big”) are 1.7 times more frequent in Hb homozygous lambs than in the Hb heterozygous individuals.

c) Association of haemoglobin genotypes with hair fibre quality (Fig. 12)

“Silky” quality of hair fibre is more common in the haemoglobin heterozygotes compared with Hb homozygotes. The “normal” aspect of hair occurs with the same frequency in both lamb subpopulations. A notable aspect for the selection process is that only these two superior characteristics of the hair fibre quality are found in the Hb heterozygous lambs, while in the haemoglobin homozygotes, in addition, some inferior characteristics of hair quality (“rough” and “soft”) appear in subpopulation, even if their frequency is low.

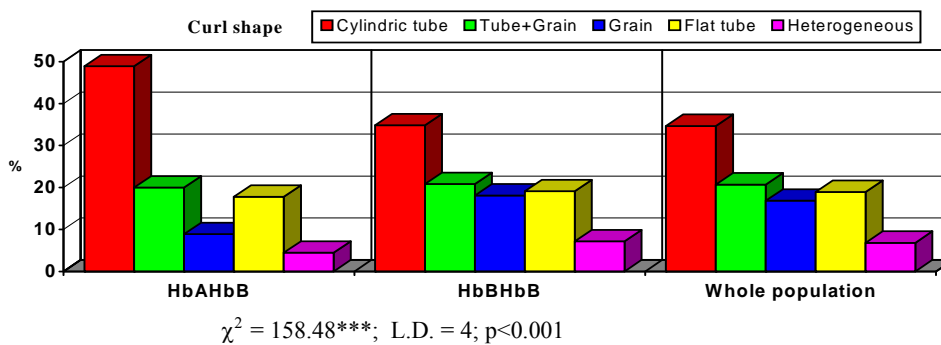


Fig. 10. Incidences of curl shapes of Botosani Karakul lamb pelts depending on haemoglobin genotypes.

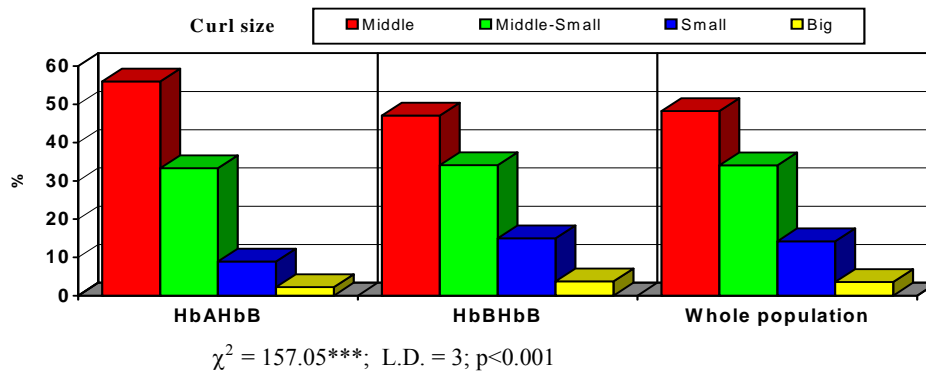


Fig. 11. Incidences of curl sizes of Botosani Karakul lamb pelts depending on haemoglobin genotypes.

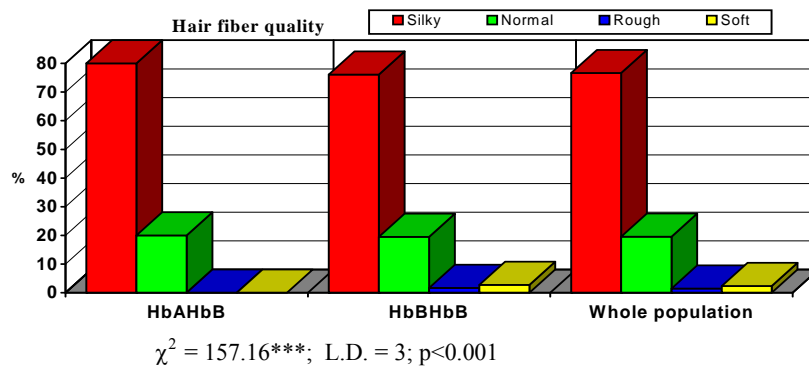


Fig. 12. Incidences of hair fibre quality of Botosani Karakul lamb pelts depending on haemoglobin genotypes.

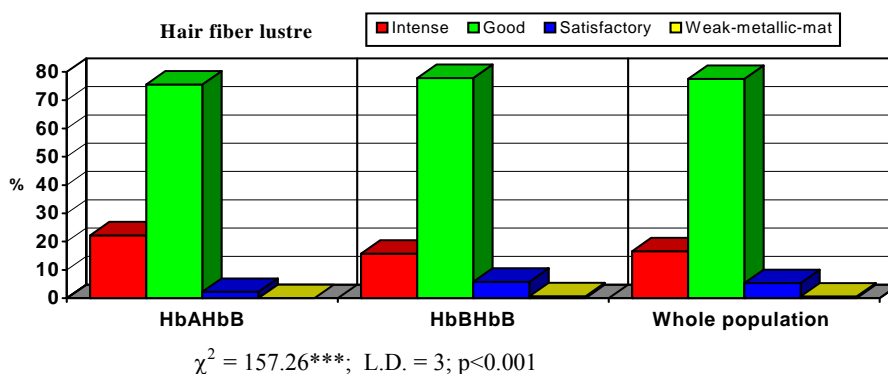


Fig. 13. Incidences of hair fibre lustre of Botosani Karakul lamb pelts depending on haemoglobin genotypes.

d) Association of haemoglobin genotypes with hair fibre lustre (Fig. 13)

As well for the previous feature of lamb pelt, the most valuable lustre of hair fibre in economic terms, the “intense” one, has a higher incidence in Hb heterozygotes than in Hb homozygotes. The other valuable characteristics of this feature, “good” lustre, is insignificantly more prevalent in homozygotes compared with heterozygotes. The “satisfactory” lustre, increasingly difficult accepted in the selection criteria, is 2.6 times more common in homozygotes than in heterozygotes. The inferior lustre, of “weak-metallic-mat” type, is found only in the haemoglobin homozygotes, although its spreading in subpopulation is sporadic.

*

Therefore, the valuable, superior characteristics from the level of any qualitative features of lamb pelts are better associated with the Hb^AHb^B heterozygous genotype than with the Hb^BHb^B homozygous genotype. The inferior, valuable characteristics are almost twice less widespread in heterozygous lambs compared with homozygous individuals at the level of some features (such as curl type, curl size and partially the hair fibre lustre) or may be missing completely in heterozygotes at other features (hair quality and partially the hair fibre lustre). The valuable features of lamb pelts are minus variants and if are not controlled, the selection and improvement objective may deviate from the intended purpose.

The superiority of the Hb heterozygotes compared to the Hb homozygotes, in terms of lamb pelt features, might be due to the different haemoglobin types found in the two lamb categories, haemoglobin types that have distinct particularities of biochemical, biophysical and physiological structure that could influence the metabolic profile parameters from the follicle level in intrauterine phase¹⁸.

Due to the better association of haemoglobin genotypes with superior features of lamb pelts in Hb heterozygous compared to Hb homozygous it is necessary to improve the genetic structure of the Botosani Karakul breed at the Hb locus by increasing the heterozygosity. This is all the more because the Botosani Karakul breed, and generally in sheep of the Karakul “macro-breed”, the Hb heterozygosity is much lower than Hb homozygosity (especially the one for Hb^B allele) or there are even obvious signs of diminishing the heterozygosity at the Hb locus if the selection does not take place with great accuracy⁹. Thus enriching the haemoglobin heterozygosity the chance increases to improve the quality of the lamb pelt features.

The comparative analysis of the two empirical distributions concerning the qualitative features of Karakul lambskins in relation to their belonging to genotypes haemoglobin shows that the two lamb subpopulations differ very significantly, given the high values of the χ^2 test, exceeding the most critical significance threshold – 0.1% (transgression probability $p < 0.001$) (Figs. 10–13).

The lamb pelt quality is given by the sum of all qualitative characteristics; the phenotyping of each feature depends on all other features that influence each other, contributing to the overall look of lamb pelt. The zoo-economic value of lambskin depends on how these features and characteristics are interrelated.

In the whole population and homozygous subpopulation at the Hb locus almost all correlations among different features of lamb pelts are very strong, very significant, most influencing each other in shaping the lamb pelts (Table 1). The exception is the correlation between the curl size and hair fibre lustre, the interconditioning between the two qualitative traits being insignificant.

Table 1

Correlations among the qualitative features of Karakul lamb pelts depending on haemoglobin genotypes on the R x C contingency table basis

Correlation type	Haemoglobin genotype				Whole population	
	Hb ^A Hb ^B		Hb ^B Hb ^B		L.D.	χ^2
	L.D.	χ^2	L.D.	χ^2		
Curl shape – curl size	12	29.88**	12	79.88***	12	96.04***
Curl shape – hair fibre quality	4	10.36*	12	42.44***	12	49.62***
Curl shape – hair fibre lustre	8	27.27***	16	108.43***	16	129.82***
Curl size – hair fibre quality	3	5.66	9	28.80***	9	33.48***
Curl size – hair fibre lustre	6	7.96	12	15.23	12	16.53
Hair fibre quality – hair fibre lustre	2	6.76*	12	147.75***	12	254.71***

In the heterozygous subpopulation at Hb locus only the correlation between the curl type and hair fibre lustre is very significant. The correlation between the curl shape and its size is distinctly significant and the one between the curl shape and hair fibre quality is significant. A significant correlation appears between the quality and lustre of hair fibre, too. The curl size does not correlate significantly with hair quality and neither with hair fibre lustre. The less intense correlations in the Hb^AHb^B lambs compared with those found in the Hb^BHb^B lambs would be due to lower or no occurrence of some features of lambskins, namely of those worthless. These differences in the qualitative aspects would have a selective advantage in livestock practice, being able to work in heterozygous subpopulation on the selection of a character without affecting another character in a so large extent as in homozygous lambs.

e) Association of haemoglobin genotypes with zootechnical classes (Fig. 14)

The zootechnical classes represent individual groups constituted according to morphological and production traits established by animal evaluation. In fact, the characteristics of zootechnical classes represent the sum of production performances of animals. In sheep specialized for lamb pelt production, which includes the Botosani Karakul breed too, the individual distribution in zootechnical classes is done by evaluating the qualitative features of lambs in the first three days after their birth. In sheep for lamb pelts there are five zootechnical classes: Record, Elite, Ist, IInd and IIIrd. Due to the advanced improvement stage of the Botosani Karakul sheep only the first three zootechnical classes are found in this breed (Fig. 14).

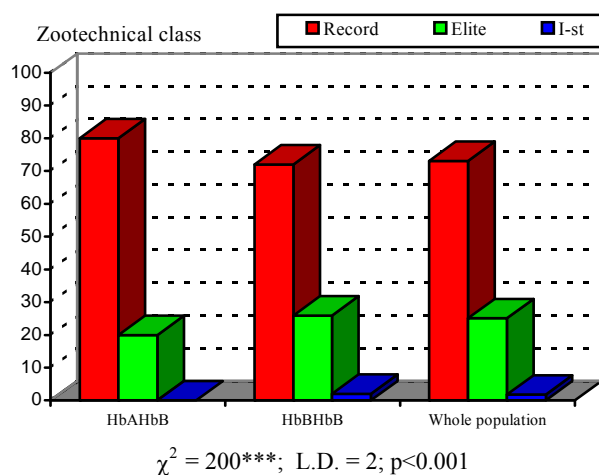


Fig. 14. Distribution in zootechnical classes of Botosani Karakul lamb pelts depending on haemoglobin genotypes.

Obviously, most lambs belong to the Record class. The Elite class has a moderate frequency and individuals in the Ist class were very rare. In terms of belonging to the haemoglobin genotypes the frequency of lambs in the Record Class is 8% higher in Hb homozygotes than in Hb heterozygotes. Within the Elite class, the homozygous lambs are 6% more frequently than those heterozygous. If in the haemoglobin heterozygotes the Ist class is absent, this class appears in homozygotes with a certain frequency (2.05%). So also in terms of lamb classification in zootechnical classes, the Hb^AHb^B heterozygotes are superior to Hb^BHb^B homozygotes as overall morphological and production performances. These productivity differences present very significant statistical assurance.

Therefore, the use of haemoglobin genotypes as genetic markers in selection and improvement works of the Botosani Karakul sheep for the lamb pelt production would be a really helpful instrument for the livestock practice of this breed.

CONCLUSIONS

The Botosani Karakul breed is characterized by a moderate polymorphism at the determinant haemoglobin locus, expressed through existence of two genotypes: Hb^AHb^B heterozygote and Hb^BHb^B homozygote; the homozygote for Hb^A allele missing from the haemoglobin panel.

In the Botosani Karakul breed lambs the valuable characteristics of the qualitative features of lamb pelts prevail; the inferior characteristics of these features occur with less frequency or the spread of some of them is sporadic.

The Hb^AHb^B heterozygotes are better associated with the valuable features of lamb pelts compared to Hb^BHb^B homozygotes; lower incidence of characteristics of the same features of lamb pelts in Hb heterozygous individuals is much lower or sometimes zero to that of Hb homozygous lambs.

The empirical distributions of qualitative features of lamb pelts in relation to haemoglobin in homozygotes (groupings) differ very significantly concerning the most important morphological and production parameter of the Botosan Karakul breed.

Generally, the qualitative features of lamb pelts are interrelated in different significance degrees; correlation intensity among these features is much stronger and more significant in Hb homozygotes in comparison with Hb heterozygotes.

The Hb^AHb^B heterozygotes are better associated with the Record class as compared to Hb^BHb^B homozygotes, while the lamb framing in the Elite class is slightly higher for homozygotes than for heterozygotes; the lambs included in the Ist class (inferior class) are found only in Hb homozygotes.

The haemoglobin genotypes in association with qualitative features of lamb pelts can be used as genetic markers in the selection, improvement and exploitation technologies of sheep to increase this production parameter of the Botosani Karakul sheep.

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