Scientific Papers. Series D. Animal Science. Vol. LXVII, No. 2, 2024
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

# COMPARATIVE RESEARCH BETWEEN PURE BREED KARAKUL AND MEAT CROSSBRED REGARDING THE FREQUENCY OF GENOTYPES AND PREDISPOSITION TO SCRAPIE

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***Abstract***

*The present work aimed to genotyping sheep in order to know the predisposition to scrapie and take the necessary measures. The analyzed biological material was represented by 822 pure breed Karakul sheep and by 239 crossbred meat sheep obtained by crosses between females of the Karakul breed and males of the Palas meat line. The method of determining the genotypes of susceptible sheep to scrapie from biological samples, consists in the analysis of the coding region of the PRNP gene (exon 3) where there are three codons associated with the resistance to this disease. Purebred Karakul sheep which have the genotypes belonging to classes 1 and 2, with the highest resistance to scrapie, have the share of 25.70%, compared to crossbred meat sheep in which the share of genotypes for the mentioned classes was 58.70% (significant for p<0.01 and C.I = 95%). An increase in resistance to scrapie was found in crossbred meat sheep which proves that by practicing infusion crosses with disease-resistant breeds, we will be able to increase scrapie resistance in the case of purebred Karakul sheep.*

***Key words****: genotyping, Karakul, resistance, sheep, scrapie.*

## INTRODUCTION

The Karakul sheep breed is specialized in the production of skins and has its origin in Asia. In 1910, a total of 160 sheep and rams were imported from Bukhara (Turkmenistan, Kazakhstan). Starting from 1948, the action of crossing the populations of Black Grouse and Greyish from the North-Eastern region of Moldova with Karakul rams was carried out. In this way, the foundations were laid for the formation of a new type of native sheep for the production of hides called Karakul de Botoșani. Currently, there are eight color lines within the breed, respectively Black, Greyish, Brown, Grey, Pink, White, Halili and Sarga. In 1988, it was approved the new sheep breed Karakul of Botoșani with two color lines: Black and Greyish. In 2010 was approved he Brown variety and, in 2018, the Grey one.

The progress registered in recent years in genome analysis technologies has allowed the identification of DNA markers responsible for the variability of phenotypic characters in sheep (Cassmann et al., 2021; McHugh et al., 2022).

The implementation of DNA testing (genotyping) in classical sheep breeding programs based on selection on phenotypic criteria of breeders, can bring important benefits in their genetic management.

The early genotyping of sheep allows for a more efficient matching of mattings, which can have the positive effect of limiting inbreeding, faster fixation of some characters of interest specific to each breed and, last but not least, maximizing genetic progress (Sacchi et al., 2018; Silva et al., 2023).

There are a multitude of genetic diseases that can affect sheep populations and which are of interest for improvement programs. The identification of nucleotide mutations that cause these genetic diseases is essential, because allows the elimination by DNA testing

the individuals who carrying mutations associated with undesirable phenotypes.

Scrapie is a lethal contagious disease, which naturally affects mainly the sheep and goats, very rarely other small herbivores. In the manifestation of disease appear characteristic of nervous disorders, caused by the presence of an abnormal prion protein in the nervous system, where it induces specific neurodegenerative lesions, with a spongy appearance. Scrapie is part of a wider group of diseases, called Transmissible Spongiform Encephalopathies - TSEs, with a common element: the appearance of degenerative spongiform lesions in the nervous system. This group of diseases, apart from scrapie, also includes: Bovine spongiform encephalopathy-BSE (mad cow disease), Chronic wasting disease, Mink transmissible encephalopathy, Feline spongiform encephalopathy. There are also several transmissible encephalopathies considered specific to humans: Kuru Disease, Creutzfeldt-Jacob Disease -BCJ, Creutzfeldt-Jacob Disease Variant-vBCJ (Vaccari et al., 2004).

Hence, the need for sheep genotyping to know the predisposition to scrapie and take the necessary measures (Hrincă et al., 2014; Coşier, 2007).

## MATERIALS AND METHODS

The biological material analyzed was represented by the active herd of pure breed Karakul 822 sheep and 239 crossbred meat sheep obtained by crosses between females of the Karakul breed and males of the Palas meat line. The entire purebred herd subject to evaluation is included in the Genealogical Register of the breed and is in the improvement program for skin quality.

The rules and responsibilities regarding the method of determining by DNA tests (exon 3 polymorphism analysis of the PRNP gene) the genotypes susceptible/resistant to scrapie from sheep from biological samples (hair, blood and other tissues), consists in the identification of the genotypes susceptible/resistant to scrapie from sheep by analyzing the entire coding region of the PRNP gene (exon 3). In exon 3 are located the three codons (136, 154 and 171) that have been associated in various studies with the resistance or susceptibility of sheep to this disease (Belt et al., 1995; Hunter et al., 1993; Goldmann et al., 1994). Amplification of exon 3 from PRNP gene is carried out from DNA samples purified from blood, hair or other tissues from sheep with the help of specific primers that flank the coding region of this exon and that generate a fragment of over 860 bp. This fragment is then subjected to a sequencing process following which the mutations present in the three codons can be identified based on the generated chromatograms (Coşier & Dărăban, 2016).

The method is applied to identify the sheep with susceptible/resistant genotypes to scrapie using samples of hair, blood or other tissues. Early genotyping of sheep at the PRNP locus allows a rapid selection of genotypes conferring resistance to this disease and subsequently has an improvement in the genetic resistance of native sheep breeds. The presence of the disease was diagnosed in native sheep breeds by histopathological examination (Cătoi et al., 2008) and the frequency of susceptible/resistant genotypes was estimated in various breeds (Coşier, 2007; Coşier et al., 2008; Hrincă et al., 2014; Coşier et al., 2011).

Sample collection is performed under sterile conditions to avoid cross-contamination. In the case of blood samples, they are collected in sterile vacutainers containing K3EDTA as anticoagulant. In the case of hair samples, they must be harvested by plucking (tissues must be harvested with a sterile scalpel) and transferred into sterile plastic tubes. Biological samples must be marked on tubes with identification numbers and will be stored at -200°C until processing in order to avoid their alteration or change in composition.

Reagents required: DNA purification kits, proteinase K, PCR amplification kits (polymerase), agarose, Tris Borate EDTA solution, gel fluorescent dye (Sybr-Safe), DNA length marker (DNA ladder), purification kits, reaction of sequencing, sequencing kits.

Required materials: sample collection tubes, Eppendorf tubes (1.5-2 ml), tips (200 ul, 1000 ul), PCR tubes (0.2 ml), sequencing plates, stands, pipettes, etc.

The required measuring and testing equipment are: DNA sequencing / genotyping laboratory: Genetic analyzer 3130xl Applied Biosystem,

Vacuum centrifuge for concentration of nucleic acid samples, centrifuge with cooling, marine bath, electronic balance.
Laboratory for receiving samples, sterilization of solutions and consumables, purification and quantification of nucleic acids: refrigeration unit, chemical hood, centrifuge with cooling, vortex, marine bath, spectrophotometer.
PCR genotyping laboratory (setting reactions / amplification): PCR hood, thermocycler, thermostat, refrigeration unit.
Bioinformatics laboratory: computer, printer.
Electrophoresis laboratory (analysis of amplification products): chemical fume hood, analytical balance, horizontal agarose gel electrophoresis, microwave oven, gel image analysis system.
The genotypes thus obtained can be classified into one of the following classes:
- Risk class 1 (R1): ARR/ARR genotypes - associated with the highest resistance to scrapie;
- Risk class 2 (R2): genotypes ARR/AHQ, ARR/ARH, ARR/ARQ - associated with an average resistance to scrapie;
- Risk class 3 (R3): genotypes AHQ/AHQ, AHQ/ARH, AHQ/ARQ, ARH/ARH, ARH/ARQ, ARQ/ARQ - associated with low resistance to scrapie;
- Risk class 4 (R4): ARR/VRQ genotypes - associated with an increased susceptibility to scrapie;
- Risk class 5 (R5): genotypes ARQ/VRQ, AHQ/VRQ, ARH/VRQ, VRQ/VRQ - highly susceptible to the onset of the disease.
Early genotyping of sheep at the PRNP locus allows rapid selection of genotypes conferring resistance to this disease and therefore allows improvement of the genetic resistance of native sheep breeds.
The data thus obtained, following the genotyping of the flock under study, were systematized and processed statistically. The statistics, respectively the parameters, which characterize a normal distribution, are on the one hand the mean or median, and on the other hand the dispersion indices represented by the variance and the standard deviation of the observed character.
For this purpose, the computer program SPSS 16.00 for WINDOWS was used to determine the frequency, the arithmetic mean ($\bar{X}$) the error of the arithmetic mean ($\pm s_{\bar{x}}$) the standard deviation (s), Chi-Square Tests, ANOVA Test, the significance test p. and the confidence interval (BUT.).
The statistical test is a decision method that helps us to validate or invalidate with a certain degree of certainty a statistical hypothesis:
hypothesis H0 (or null hypothesis): the data are not related, they are independent/the compared values do not differ from each other hypothesis H1 (or alternative hypothesis): the data are related, dependent/the compared values differ from each other

## RESULTS AND DISCUSSIONS

Regulation (EU) no. 630/2013 of the Commission of June 28, 2013 amending the annexes to Regulation (EC) no. 999/2001 of the European Parliament and of the European Council establishing regulations for the prevention, control and eradication of certain transmissible forms of spongiform encephalopathy, regulates and shows that the objective of the breeding program is to increase the frequency of the ARR allele within the herd, reducing, in at the same time, the prevalence of alleles whose contribution to TSE susceptibility has been demonstrated. The same normative act emphasizes the obligation of individual identification by secure means of the animals in the herd that are to be subjected to a genotyping test.
Therefore, the results obtained from genotyping the active Karakul purebred herd are presented in Table 1.

Table 1. Genotypes of Karakul purebred sheep

| Genotip | | Frequency | Percent | Valid Percent | Cumulative Percent |
|---|---|---|---|---|---|
| Valid | AHQ/ARQ | 10 | 1.2 | 1.2 | 1.2 |
| | ARH/ARH | 5 | .6 | .6 | 1.8 |
| | ARH/ARQ | 71 | 8.6 | 8.6 | 10.5 |
| | ARH/VRQ | 1 | .1 | .1 | 10.6 |
| | ARQ/ARH | 1 | .1 | .1 | 10.7 |
| | ARQ/ARQ | 513 | 62.4 | 62.4 | 73.1 |
| | ARQ/VRQ | 11 | 1.3 | 1.3 | 74.5 |
| | ARR/AHQ | 1 | .1 | .1 | 74.6 |
| | ARR/ARH | 11 | 1.3 | 1.3 | 75.9 |
| | ARR/ARQ | 180 | 21.9 | 21.9 | 97.8 |
| | ARR/ARR | 14 | 1.7 | 1.7 | 99.5 |
| | ARR/VRQ | 1 | .1 | .1 | 99.6 |
| | Abnormal | 3 | .4 | .4 | 100.0 |

| Genotip | | Frequency | Percent | Valid Percent | Cumulative Percent |
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| | ARH/ARQ | 71 | 8.6 | 8.6 | 10.5 |
| | ARH/VRQ | 1 | .1 | .1 | 10.6 |
| | ARQ/ARH | 1 | .1 | .1 | 10.7 |
| | ARQ/ARQ | 513 | 62.4 | 62.4 | 73.1 |
| | ARQ/VRQ | 11 | 1.3 | 1.3 | 74.5 |
| | ARR/AHQ | 1 | .1 | .1 | 74.6 |
| | ARR/ARH | 11 | 1.3 | 1.3 | 75.9 |
| | ARR/ARQ | 180 | 21.9 | 21.9 | 97.8 |
| | ARR/ARR | 14 | 1.7 | 1.7 | 99.5 |
| | ARR/VRQ | 1 | .1 | .1 | 99.6 |
| | Abnormal | 3 | .4 | .4 | 100.0 |
| | Total | 822 | 100.0 | 100.0 | |

Analyzing the information in table 1, we find that sheep with genotypes ARH/VRQ, ARQ/VRQ, ARR/VRQ belonging to classes 4 and 5 with increased and very increased susceptibility to scrapie represent a weight of 1.50%, which represents 13 heads of the total 822 sheep. These sheep have restrictions on sale or breeding and must be slaughtered in 6 months maximum.

Sheep of the pure breed Karakul with genotypes belonging to class 3, associated with low resistance to scrapie have a weight of 72.80% and represent 599 sheep. No sales or breeding restrictions apply to these sheep.

A weight of only 25.70% represents sheep belonging to classes 1 and 2 with the highest and average resistance to scrapie.

Practically, it can be assumed that the classic form of scrapie is spread all over the world at the present time, the only countries considered free of scrapie being Australia and New Zealand, although the disease was reported in them in 1950. According to the O.I.E., if in a country in which epidemiological surveillance is officially practiced and no new cases of scrapie have been reported in the last 7 years, that country can be considered free.

In our country, the first outbreak of scrapie was diagnosed in 2003, within IDSA by Dr. Alexandru Nicolae. Later, other outbreaks were identified in several other counties. The causes underlying the appearance of the disease and its pathogenesis are not completely and definitely elucidated even today. This is because, although it can still be considered an infectious-contagious disease, like most microbial diseases, it clearly differs from all of them in that it is not produced by any bacteria or virus, but by an isomer of a normal protein from the body, without the participation of any nucleic acid (DNA or RNA) to ensure its multiplication. The possibility of transmission of the disease to sheep and goats in natural conditions, as well as experimental transmission to other species, were proven long after the description of the disease. The pathogenesis, completely particular, complex and complicated, was partially deciphered later on and as the accumulated results of the studies performed in parallel in the other spongiform encephalopathies, of humans and animals.

The distribution of colors in the Karakul purebred herd taken in the study is shown in Table 2.

Table 2. Absolute and relative frequency for colour at Karakul breed

| Colour | | Frequency | Percent | Valid Percent | Cumulative Percent |
|---|---|---|---|---|---|
| Valid | White | 88 | 10.7 | 10.7 | 10.7 |
| | Greyish | 80 | 9.7 | 9.7 | 20.4 |
| | Comor | 6 | 0.7 | 0.7 | 21.2 |
| | Halili | 25 | 3.0 | 3.0 | 24.2 |
| | Brown | 77 | 9.4 | 9.4 | 33.6 |
| | Black | 257 | 31.3 | 31.3 | 64.8 |
| | Pink | 96 | 11.7 | 11.7 | 76.5 |
| | Şarg | 1 | 0.1 | 0.1 | 76.6 |
| | Grey | 192 | 23.4 | 23.4 | 100.0 |
| | Total | 822 | 100.0 | 100.0 | |

The black color in the purebred Karakul has the highest weight of 31.30% and is followed by the yellow color with a weight of 23.40%. The explanation lies in the greater market demand for black skins.

The total score for purebred Karakul sheep is shown in Table 3.

Table 3. Total points at Karakul purebred sheep

| Descriptive Statistics | N | Minimum | Maximum | Sum | Mean | | Std. Deviation | Variance |
|---|---|---|---|---|---|---|---|---|
| | Statistic | Statistic | Statistic | Statistic | Statistic | Std. Error | Statistic | Statistic |
| Total points | 822 | 390.00 | 690.00 | 428417.00 | 521.1886 | 1.93579 | 55.50020 | 3080.273 |
| Valid N (listwise) | 822 | | | | | | | |

Analyzing the information in the table, we find an average value of 521.18 points with limits between 390 and 690 points. The standard deviation from the mean is 55.50 points.

The results regarding the genotyping of the crossbred meat sheep flock, obtained from crosses between Karakul breed females and Palas meat line males, are presented in Table 4 and Figure 1.

Analyzing the values in Table 4, it can be seen that crossbred meat sheep with genotypes in classes 4 and 5 with increased and very increased susceptibility to scrapie have a weight of 1.20%, and 40.10% represent sheep with genotypes in class 3 associated with an average resistance to scrapie. Correspondingly, classes 1 and 2 with sheep whose genotypes are associated with the highest and average resistance to scrapie have a weight of 58.70% and 140 sheep out of the total of 239 crossbred sheep studied.

So, we see an increase in resistance to scrapie in crossbred meat sheep obtained by crosses between Karakul females and Palas meat line males. From this, we can conclude that by practicing infusion crosses with breeds well analyzed in terms of disease resistance, we will be able to increase the resistance to scrapie in the case of purebred Karakul sheep. Hides as the main production of the Karakul breed must be preserved and preserved, but improvement of milk and meat production is absolutely necessary

Table 4. Genotypes at crossbred meat sheep

| Genotypes | | Frequency | Percent | Valid Percent | Cumulative Percent |
|---|---|---|---|---|---|
| Valid | ARH/ARQ | 13 | 5.4 | 5.4 | 5.4 |
| | ARQ/ARQ | 83 | 34.7 | 34.7 | 40.2 |
| | ARQ/VRQ | 1 | 0.4 | 0.4 | 40.6 |
| | ARR/ARH | 6 | 2.5 | 2.5 | 43.1 |
| | ARR/ARQ | 105 | 43.9 | 43.9 | 87.0 |
| | ARR/ARR | 28 | 11.9 | 11.9 | 98.7 |
| | ARR/VRQ | 2 | 0.8 | 0.8 | 99.6 |
| | Genotype | 1 | 0.4 | 0.4 | 100.0 |
| | Total | 239 | 100.0 | 100.0 | |

Programs to improve the genetic resistance of sheep to scrapie aim to increase the frequency of ARR/ARR type genotypes through genotyping and their promotion in reproduction.

Participation in breeding programs has so far been limited to flocks of sheep of high genetic value. In cases where have been applied, the breeding programs have been effective in increasing resistance to classical scrapie in sheep populations of high genetic value. But the diffusion, within the production populations, of the hereditary factor (allele) which provides the resistance seems to have been, until now, limited. Appendice VII, chapter C of Regulation (EC) no. 999/2001 should allow the genotyping of breeding rams from sheep flocks that are not participating in the breeding program to facilitate a better diffusion of the classical scrapie resistance factor within production populations.

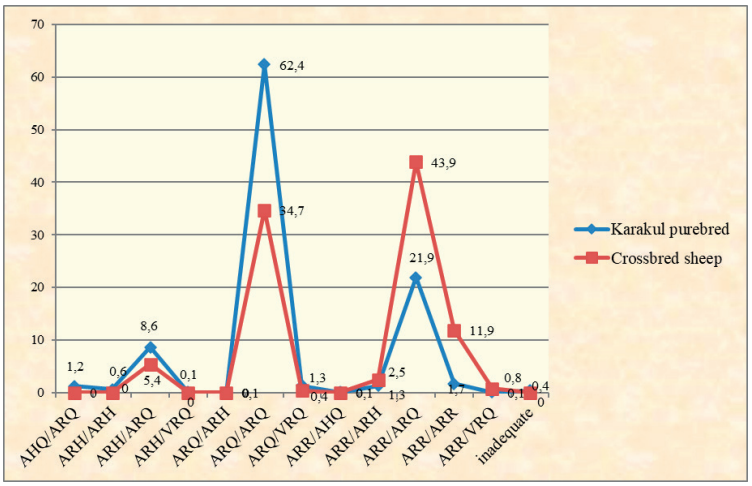


Figure 1. The relative frequency for the genotypes in the studied sheep

From Figure 1, it is evident the increase of the proportion of genotypes with resistance to scrapie in the case of the crossbred sheep for meat studied and the disappearance of some existing genotypes in the Karakul pure breed. The genetic resistance of sheep to transmissible spongiform encephalopathy (scrapie), a disease with a proven hereditary substrate, currently represents one of the main selection criteria of sheep breeders.

## CONCLUSIONS

After carrying out the study on the frequency of genotypes and the predisposition to scrapie of sheep of the pure Karakul breed and meat crossbreeds, we can conclude:

1. Sheep of the pure Karakul breed that have the genotypes belonging to classes 1 and 2 with the highest and average resistance to scrapie have the weight of 25.70%, compared to crossbred meat sheep in which the weight of genotypes for the mentioned classes was 58, 70%.
2. In the case of Karakul de Botoşani sheep, it is desirable that scrapie resistance is induced following the selection and use for breeding of individuals with ARR/ARR genotypes.
3. An increase in resistance to scrapie was found in crossbred meat sheep obtained by crosses between Karakul females and Palas meat line males, which proves that by also practicing infusion crosses with disease-resistant breeds, we will be able to increase scrapie resistance.
4. Programs to improve the genetic resistance of sheep to scrapie aim to increase the frequency of ARR/ARR type genotypes through genotyping and their promotion in reproduction.

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