SUMMARY

of the doctoral thesis entitled:

RESEARCH ON THE SELECTION OF SHEEP BREEDERS FOR SCRAP RESISTANCE

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The main aim of the present research was to identify and evaluate the relevant genetic and phenotypic criteria for the effective selection of breeders showing increased resistance to scrapie. The study aims to contribute to improving the health and resilience of sheep flocks by reducing the prevalence of disease, thus ensuring increased productivity and sustainability of sheep populations. Breeding for scrapie resistance is a key priority for genetic improvement and sustainable sheep breeding.

This process improves animal health and helps protect public health by reducing the risk of disease transmission. Scrapie is a fatal neurodegenerative disease affecting sheep and goats, having a significant impact on animal health and the livestock industry. In this context, research has focused on the identification and evaluation of prion protein polymorphisms associated with resistance or susceptibility to scrapie. The exploration of these polymorphisms has allowed the development of effective strategies for the selection of male breeders, strategies that we hope will thus ensure the long-term viability of the herds.

Another important objective of the research was the development and implementation of new, effective strategies for the selection of scrapie-resistant breeders, by creating an effective genetic selection protocol based on the one hand on the analysis of identified polymorphisms, but which can be applied in practice by sheep farmers. The paper proposes a detailed and practical protocol for the selection of male breeders based on prion protein polymorphisms, which can be implemented by sheep breeders. This protocol uses advanced statistical modeling techniques to observe the probability of each breeder to produce resistant progeny, specifically using the thresholded biometric model, the potential to significantly improve herd resistance to scrapie. By using the biometric threshold model, this study uniquely addresses the problem of breeder selection for scrapie resistance.

By integrating the results obtained in the existing theoretical context and by applying them in practice, the study makes a significant contribution to the understanding of the genetic mechanisms of resistance to scrapie and to the improvement of sheep flocks. These contributions can serve as a starting point for

further research and the development of innovative solutions in the field of animal genetics.

CHAPTER VI. ANALYSIS OF PRION PROTEIN POLYMORPHISMS IN THE FRAMEWORK OF SHEEP BREEDS RAISED IN ROMANIA

In this chapter VI, the study focused on the identification and evaluation of prion protein polymorphisms (PRNP) associated with susceptibility and resistance to scrapie in sheep breeds raised in Romania. Analysis of samples from the years 2019 and 2020 revealed that of the 15 possible combinations of alleles, only 13 were expressed in each year. ARH/ARH homozygous and AHQ/ARH heterozygous genotypes were absent in 2019, and ARQ/ARH and ARQ/AHQ heterozygous genotypes were not present in 2020. Constant and slightly increased presence of ARR/ARR homozygous allele, from 16.8% in 2019 to 17.98% in 2020, and the abundance of the ARR/ARQ heterozygous genotype (38.79% in 2019 and 40.30% in 2020) underlines the breeding preferences for scrapie-resistant genotypes.

Within the Karakul sheep population, data analysis included, in addition to progeny genotypes, calving weight and coat color. The frequency of ARR, ARH, ARQ, VRQ and AHQ alleles was shown, with ARQ being the most prevalent, followed by ARR and AHQ.

The VRQ allele had a very low incidence, emphasizing the need for increased surveillance to manage the risk of scrapie transmission.

The Sanger sequencing method was used for scrapie susceptibility screening, providing high accuracy in identifying specific PRNP gene polymorphisms. This has facilitated the genetic selection and management of sheep flocks, contributing to the development of effective genetic breeding strategies.

Genotype frequency analysis between 2019 and 2020 showed significant stability, with 13 of the 15 possible allele combinations expressed each year. The constant and slightly increased presence of the homozygous ARR/ARR allele and the predominance of the heterozygous ARR/ARQ genotype reflect a tendency to prioritize genotypes with high resistance to scrapie.

The ARQ allele, associated with minimal susceptibility to scrapie, was the most prevalent, present in 120 occurrences in the study population and with 622 carrier individuals. In contrast, alleles associated with resistance, such as ARR and AHQ, were present at lower frequencies, underscoring the existing genetic diversity.

The VRQ allele had a very low incidence, being observed in only 4 individuals. This highlights the need for increased surveillance and monitoring efforts to manage the risk of scrapie transmission and to implement proactive management strategies.

The Sanger sequencing method has proven to be extremely useful in scrapie susceptibility screening, providing high accuracy in identifying specific PRNP gene polymorphisms. This contributes significantly to the development of effective genetic improvement strategies and to the reduction of scrapie in sheep populations.

The results of this study are consistent with those reported by Hrinca et al. (2014), who associated the ARQ allele with minimal susceptibility to scrapie. The predominance of this allele in the Romanian sheep population suggests a low risk of scrapie transmission, similar to their conclusions.

CHAPTER VII. PHENOTYPICAL CHARACTERIZATION OF THE STUDIED POPULATION

In this chapter, the phenotypic characteristics of several breeds of sheep raised in Romania were explored in detail, evaluating both scrapie resistance and other essential parameters for animal health and productivity. The study included detailed analysis of the Turkana, Șigaie, Merino and Karakul breeds.

The Turcan breed presented a remarkable genetic diversity, with the predominance of ARQ/ARQ (53.50%) and ARR/ARQ (37.85%) genotypes. The ARQ/ARQ genotype is associated with moderate susceptibility to scrapie, while the ARR/ARQ genotype indicates greater resistance. Phenotypic characteristics include wool production (2.2 kg for ewes and 3.2 kg for rams), milk production (average 70-90 liters with a maximum of 235 liters), and average daily gain (140-160 g/head/day) .

The Șigaie breed is mainly characterized by ARQ/ARQ (50.65%) and ARR/ARQ (39.57%) genotypes, suggesting moderate susceptibility and higher resistance to scrapie, respectively. Phenotypically, the Tiger has a body weight at birth of 3-4.5 kg and an adult body weight of 45-47 kg. Wool production is 4-5 kg for sheep and 6-8 kg for rams, and total milk production is 150-170 liters.

The Merino breed, known for its excellent wool production, has the predominant genotypes ARR/ARR (42.22%) and ARR/ARQ (45.81%), indicating good and moderate resistance to scrapie. Phenotypically, wool production varies between 6-8 kg for ewes and 12-14 kg for rams. Milk production is 130-160 liters, and the average daily gain exceeds 200 g.

The Karakul breed stands out for its adaptability and robustness. The distribution of alleles in the Karakul population includes ARR (1.7%), ARQ (88.6%), ARH (8.1%), and AHQ (1.6%). Body weight at calving is 4.47 kg, and adult weight varies between 40-45 kg for ewes and 70-80 kg for rams. Wool production is 1.8-2.5 kg. The predominance of the ARQ allele suggests the need for genetic interventions to increase scrapie resistance.

Analysis of genotypes between 2019 and 2020 showed considerable stability, with a tendency to prioritize genotypes with high scrapie resistance, such as ARR/ARR and ARR/ARQ. The studied breeds showed moderate to high genetic diversity, with the predominance of ARQ and ARR alleles, suggesting a favorable potential for genetic improvement directed towards scrapie resistance. The predominance of the ARQ allele in some breeds, such as the Karakul, underlines the need for rigorous genetic selection programs to reduce susceptibility to scrapie.

Calving weight, wool production and milk production are essential phenotypic parameters that reflect the health and viability of the studied populations, being relevant for management strategies and genetic selection.

CHAPTER VIII. ESTIMATION OF GENETIC PARAMETERS FOR SCRAP RESISTANCE

Analysis of genetic parameters for scrapie resistance revealed significant differences between the various sheep breeds studied, using the linear mixed model emREML. Turcana and Merino breeds showed the highest values of additive variance, indicating a considerable genetic potential for these breeds in terms of scrapie resistance. The residual variance was higher in the Șigaie and Cap Negru de Teleorman breeds, and the total variance was the highest in the Turcană and Merino breeds, reflecting a high overall variability in these populations.

The Turcan and Merino breeds had the highest heritability values (0.925 and 0.801, respectively), suggesting that scrapie has a genetic determinism that varies from highly heritable in these breeds. In contrast, the lower values of heritability in the Awassi (0.194) and Ille de France (0.31) breeds indicate a weak genetic determinism in these breeds.

The distribution of alleles in the Karakul population showed a predominance of the ARQ allele (88.6%), indicating the need for genetic interventions to increase the frequency of the ARR allele, associated with increased resistance to scrapie. By comparison with other studies that have also attempted to establish the genetic determinants of scrapie, Houston et al. (2003) used quantitative trait locus (QTL) mapping and genome-wide association studies (GWAS) to estimate the heritability of susceptibility to scrapie, highlighting the importance of identifying regions of the genome associated with resistance. Although the methodological approaches differ, both studies emphasize the importance of genetic selection in improving scrapie resistance.

The lack of detailed information on the complete pedigree of individuals may bias estimates of genetic parameters, highlighting the need for more complete data for future analyses. The environment in which animals are reared can significantly influence the manifestation of phenotypes, adding variability to estimates. Further studies should consider different environmental conditions and management practices. The heritability values obtained in this study are indicative and should not be used as definitive reference values without further validation. Further studies including a wider range of environmental conditions and more detailed data on parents and offspring are needed.

CHAPTER IX. ASSOCIATION OF GENOTYPES WITH PRODUCTION CHARACTERS

Analysis of the data in Table 9.1. revealed that birth weight and coat color are significantly correlated with productive performance of Karakul sheep, while individual genotypes and lamb score did not show significant correlations. The model used explained only a small part of the total variation in the data, suggesting that other factors, not included in the analysis, may influence the productive performances. These results highlight the need for further research to identify other genetic and environmental factors that contribute to variation in productive performance.

Birth weight had a significant positive correlation with the productive performance of Karakul sheep, suggesting that this parameter is an important predictor, while individual genotypes and lamb score did not show significant correlations with productive characters, indicating a negligible influence on productive performance and suggesting that the model used explained only a small part of the total variation in the data, with other factors not included in the analysis influencing these performances.

There is a need to identify other genetic and environmental factors that contribute to variation in productive performance, including nutrition, environmental conditions and management practices. Future studies should explore these influences using larger and diverse datasets to develop more precise and effective genetic management strategies, thereby improving the health and productivity of sheep populations. By comparison with other studies that tried to discover the relationship between scrapie and production characters, Compared to other studies, such as those carried out by De Vries et al. (2004), Hanrahan et al. (2008) and Moore et al. (2009), our results are consistent with the idea that the association between PrP genotypes and productive performance may vary considerably between different breeds. These studies explored the complex relationship between PrP genotypes and animal performance, highlighting variability between breeds and rearing conditions. Studies by Swalha et al. (2007) showed that birth weight significantly influences survival characteristics but does not explain associations between survival and PrP genotypes.

In the absence of scrapie infection, the scrapie-susceptible ARQ allele may confer greater viability, explaining its persistence in sheep populations. Also, Swalha et al. (2007) observed that susceptible PrP genotypes had lower survival in scrapie-affected herds, but could not directly link the increased mortality to the form of preclinical scrapie.

CHAPTER X. ESTIMATION OF IMPROVEMENT VALUE FOR SCRAP RESISTANCE

The biometric threshold model uses an unobservable continuous variable (liability) to determine the response category for scrapie resistance based on progeny genotypes. The study evaluated 212 progeny, born from 212 ewes mated to 52 sires, in three ordinal categories of resistance. For example, sire ram T001 had two progeny genotyped as ARQ/ARQ, falling into response category 2, indicating moderate resistance, and ram T003 had 3 progeny in 3 different production years, all with the same genotype but with distinct performance . After the data reached convergence, expressed at the final iteration, the solutions for the fixed effects (calendar year, mothers' age, sex, etc.) and the solutions for the genetic effect of the rams were obtained.

The solutions table shows the thresholds and solutions for fixed and genetic effects over six iterations used to classify individuals for scab resistance. The fixed effects of production year and maternal age vary, indicating different influences on resistance, while offspring sex has a similar influence for males and females. The genetic effects of sires vary, reflecting differences in the transmission of resistance. To calculate the response probabilities in a resistance category, the thresholds and coefficients from the last iterations are used, thus obtaining probabilities for the scrap resistance categories.

The results in the table highlight the need to prioritize rams with high probabilities of producing Category 1 progeny for improving scrapie resistance. Genotypes in Category 2 should be managed carefully and those in Category 3 should be removed from selection. Most progeny fall into Category 2, indicating moderate resistance, and the proportion of Category 3 progeny is very low, which is favorable for the breeding program. Thus, breeding strategy should focus on maximizing progeny with high resistance by using Category 1 rams and carefully managing Category 2 rams.

Finally, all genotype distribution probabilities for each sire across all years, ages and sexes were calculated and presented in Table 11.3.6. The majority of sires accepted for breeding showed a high probability of producing scrapie-resistant progeny, with the majority of progeny falling into Category 2 with moderate resistance, but also a significant proportion of highly resistant progeny (Category 1). For example, rams T029 and T027 have probabilities of 52.96% and 50.08% respectively to produce progeny in Category 1, making them ideal for breeding programs.

On the other hand, Category 3, which includes genotypes with high susceptibility to scrapie, has lower probabilities, which is positive. However, rams such as T004 have a 13.59% probability of progeny in this category, highlighting the need to remove these rams from selection programs to reduce the risk of scrapie. Table 11.3.7 shows a different distribution of probabilities, with rams T047 and T030 having lower probabilities for Category 1 and higher probabilities for Category 2. Rams T001 and T004 also have higher probabilities for Category 3, suggesting an increased risk of to produce offspring susceptible to scrapie. Therefore, genetic selection should prioritize rams with high probabilities in Category 1 and avoid rams with high probabilities in Category 3. This contrast emphasizes the importance of rigorous selection for improving the overall health and scrapie resistance of sheep flocks.

Statistical analysis of the data using the biometric threshold model demonstrated that rams with high probabilities in Category 1, such as T029 (52.96%) and T027 (50.08%), should be prioritized in breeding programs because they have a high chance to produce progeny with very high resistance to scrapie, thus representing an essential strategy for the general improvement of resistance to this disease in the sheep population. Although Category 2 contains genotypes with moderate resistance that contribute to genetic diversity, it is crucial that the breeding program focuses on phasing out these genotypes in favor of Category 1. In contrast, Category 3 rams such as T008 (2.61%) and T013 (1.61%), should be removed from selection to minimize the risk of transmission of scrapie susceptibility.

A high intensity of selection for scrapie resistance can increase the number of resistant individuals in the population, but it can also have a negative impact on other important economic traits such as calving weight, coat color and performance evaluation score. Thus, selection for scrapie resistance must be well balanced, closely monitoring production traits and maintaining genetic diversity to ensure an optimal balance between scrapie resistance and overall production performance.

The use of the thresholded biometric model allowed a detailed analysis of categorical scrapie resistance characters, facilitating a more precise assessment of genetic influences on resistance to this disease. Analysis of data collected from 212 progeny mated to 52 sire rams revealed variability in scrapie resistance influenced by both genetic and environmental factors. Fixed effects of maternal age and year of production, along with genetic effects of sire rams, contributed significantly to this variability. The results emphasize the importance of integrating these factors into genetic breeding programs to optimize selection and improve the overall resistance of sheep flocks to scrapie.