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STUDY OF THE ASSOCIATION FOR SUSCEPTIBILITY TO SCRAPIE WITH INFLUENCE ON PRODUCTIVE PERFORMANCE IN THE KARAKUL SHEEP BREED

Petrut-Lucian PARASCHIVESCU, Murshedi Osamah Mahmood ABDULZAHRA, Horia GROSU

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: petru.paraschivescu@yahoo.ro

Abstract

Scrapie, a transmissible spongiform encephalopathy, is known to be influenced by certain PRNP genotypes, and some research has shown that these genotypes can impact the productive performance of affected sheep populations. The study was conducted on Karakul sheep populations. The main aim of this work was the analysis between PRNP genotypes and productive characters (body weight, coat color etc.). Based on the breeding values, the retention of the most valuable individuals for reproduction is to be carried out according to the desired proportion of retentions. From the data analysed,none unfavorable association was observed between the PRNP allele, type, or reproductive traits. It is important to assess the wider context in which numerous variables interact to influence the productive performance of sheep populations. More studies are needed to better understand the complexities of this connection.

Key words*: genotype, improvement, productive performance, scrapie susceptibility.*

INTRODUCTION

The exploration of nonconventional transmission agents (NCTA) stands as a captivating domain within biomedical inquiry, as highlighted by Petit and Boucraut-Baralon in 1992 (Petit & Boucraut-Baralon, 1992). This area delves into infectious agents responsible for acute transmissible spongiform encephalopathies (TSEs), a class of degenerative maladies affecting the central nervous system. While the identification of such agents has been met with some contention, their potential role in instigating spongiform encephalopathies remains a subject of intense scrutiny (Petit & Boucraut-Baralon, 1992; Hunter, 1999; O'Brien et al., 2017).

TSEs manifest clinically with distinctive features, including prolonged incubation periods spanning months, years, or even decades, coupled with a gradual, afebrile progression leading to symptoms such as ataxia, tremors, and abnormal postures. Importantly, TSEs uniformly culminate in fatality. Pathologically, the hallmark lesions are evident in the gray matter of the central nervous system, characterized by neuronal sponginess and vacuolar degeneration (Somerville et al., 2002).

From an epidemiological standpoint, these maladies occasionally exhibit epizootic patterns, though primarily they are inheritable with elements of transmissibility. Notable contributions to the understanding of TSEs have been made by researchers like Hunter (1999) and Somerville (2002), shedding light on their complex etiology and epidemiological dynamics (Petit & Boucraut-Baralon, 1992; Hunter, 1999; Somerville, 2002).

Central to the discourse on TSEs is the concept of prions, entities resembling transmissible infectious agents composed of misfolded proteins. This notion underpins the Prion Hypothesis, a topic that continues to evoke debate and intrigue within the scientific community. The distinctive nature of prions lies in their structural deviation from conventional infectious agents, which typically rely on nucleic acids such as DNA or RNA. These traditional agents encompass viruses, bacteria, fungi, and parasites. In 1982, Prusiner introduced the concept of prions, coining the term as a fusion of "protein" and "infection", abbreviated as "proteinaceous infectious

particle". Prusiner's groundbreaking work proposed that aberrant forms of otherwise innocuous proteins possess the capacity to propagate disease and function as infectious agents. For his contributions, Prusiner was awarded the Nobel Prize in Physiology or Medicine in 1997, solidifying the significance of his studies on prions (Prusiner, 1982; 1991; 1998).

Prions have been implicated in diseases affecting both humans and various animal species, as elucidated by researchers like Hunter (1999) and Belay (1999). Notably, the agent responsible for scrapie disease in sheep is a prion. Given its impact on sheep, goats, and mouflons, scrapie remains classified as a prion disease primarily affecting small ruminants (Beringue & Andreoletti, 2014). Scrapie, due to its pathological implications, has spurred comprehensive research efforts encompassing biological, economic, and ecological dimensions (Beringue & Andreoletti, 2014).

Recent years have witnessed a surge in the investigation of prion protein molecular genetics within Western European sheep breeds. This uptick can be attributed to industrial crossbreeding practices aimed at developing new, highly productive sheep varieties to meet escalating market demands, particularly for meat (Fediaevsky et al., 2008). In Romania, recent endeavors have focused on breeds like Merino, Tsigai, and Tsurcana, primarily geared towards milk or meat production (Cosier, 2008; Otelea et al., 2011). Notably, the Botosani Karakul breed, renowned for its lamb pelt production, has garnered attention through specialized research initiatives. A collaboration between the Research - Development Station for Breeding of Sheep and Goats Popăuţi -Botoşani and the USAMV of Bucharest has facilitated a unique approach to studying prion-related aspects within the Karakul breed, particularly in the context of pelt production (Kevorkian et al., 2011; Hrincă et al., 2014).

MATERIALS AND METHODS

The investigation took place at Research-Development Station for Breeding of Sheep and Goats Popăuți - Botoșani, Blood samples were collected from a Karakul flock. DNA was extracted and subjected to amplification using the Real-Time PCR technique. Melting curve analysis facilitated the determination of scrapie resistance-related genotypes for each animal, specifically at the PrP locus's codons 136, 154, and 171. The process employed kits such as "LightCycler FastStart DNA Hybridization Probe MasterPLUS" (Roche Applied Science) and "LightCycler Scrapie Susceptibility Mutation" (TIB MOLBIOL), following the manufacturer's protocols (Kevorkian, 2010; Kevorkian et al., 2011). Real-time PCR experiments were conducted using the Light Cycler 2.0 instrument (Roche, 1999) with the following procedure: initial pre-incubation at 95°C for 8 minutes, followed by 45 cycles of amplification, melting curve analysis, and cooling to 40°C for 30 seconds. Each amplification cycle comprised three phases: denaturation at 95°C for 10 seconds, annealing at 60°C for 10 seconds, and extension at 72°C for 15 seconds. The melting curve analysis consisted of three steps: pre-incubation at 95°C for 2 minutes, followed by 45°C for 30 seconds, and a gradual temperature increase from 45°C to 75°C at a rate of 0.2°C per minute (Kevorkian, 2010; Kevorkian et al., 2011; Hrincă et al., 2014).

RESULTS AND DISCUSSIONS

Data obtained from the analysis of the flock include, in addition to the genotypes of the offspring, the weight at birth and the coat color, thus we tried to include these data in an animal model to observe the influence of genotypes on productive traits, or the influence of productive traits on genotypes. To estimate the genetic parameters for the weight at birth and for the shade of the coat, we used a mixed linear model emREML model (Equation 1, 2), and R-Studio was used for computing the data.

In conducting an in-depth analysis of the dataset, we employed linear modeling techniques to explore potential correlations between genotypes and individual performances. The results of this analysis were elucidated through regression analysis, facilitating a comprehensive understanding of the interplay between genetic factors and performance metrics (as summarized in Table 3 and visualized in Equation 3).

Building upon prior research efforts (De Vries et al., 2004; Hanrahan et al., 2008; Moore et al., 2009), which have delved into the complex relationship between PrP genotypes and animal performance, it became evident that this association exhibits considerable variability across different breeds.

In our study, we investigated the allelic distribution in Karakul flock, focusing on five key alleles: ARR, ARH, ARQ, and AHQ (Figure 1). All animals are resistant since alleles from R4, and R5 are absent. The frequency of each allele, along with the corresponding number of individuals harboring them, was meticulously analyzed to elucidate their potential implications in scrapie susceptibility. The most prevalent allele observed was ARQ, constituting 120 occurrences in the population, with a substantial number of individuals (622) carrying this allele.

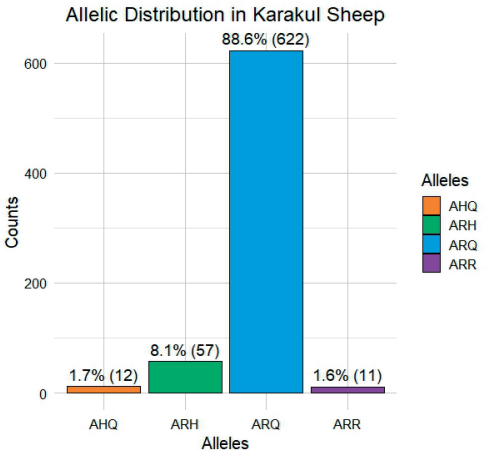

Figure 1. Allelic distribution in Karakul sheep (original)

The predominance of the ARQ allele aligns with previous research associating it with minimal susceptibility to scrapie (Hrinca et al., 2014). Its high frequency suggests minimum risk of scrapie transmission within the population, and a high resistance among rams. Conversely, alleles associated with resistance to scrapie, such as ARR and AHQ, were present in relatively lower frequencies. Specifically, the ARR allele was identified in 50 instances, followed by AHQ with 20 occurrences. Despite their lower prevalence, the existence of these resistant alleles underscores the genetic diversity within the population, offering potential avenues for selective breeding aimed at enhancing resistance to scrapie.

Notably, the ARH allele, often considered neutral regarding scrapie susceptibility, was observed in 30 instances. While its frequency is moderate compared to ARQ, its presence contributes to the genetic heterogeneity of the population, influencing the overall resistance dynamics.

However, it's noteworthy that existing investigations have predominantly centered around a limited selection of breeds, thereby warranting further exploration across a broader spectrum of genetic backgrounds.

In the realm of sheep health parameters, the linkage between PrP genotype and various health indicators has been relatively underexplored. While some studies have focused on parameters such as somatic cell count or mastitis (De Vries et al., 2004; Ligios et al., 2005), a comprehensive understanding of the broader health implications of different genotypes remains elusive. Moreover, despite the lack of a clear correlation between scrapie genotype and perinatal lamb survival, there exists a notable gap in our understanding regarding the potential influence of scrapie genotype on lambing characteristics. Existing research on this front is scarce, and further investigations are warranted to elucidate any potential relationships (De Vries et al., 2004; Ligios et al., 2005).

Of particular interest is the connection between scrapie genotype and postnatal lamb survival beyond the critical 24-hour threshold following delivery. Despite inconsistent findings in this domain, the study by Sawalha et al. (2007) shed light on higher postnatal lamb survival rates among Scottish Blackface lambs with the ARQ haplotype compared to other genotypic variants.

However, such insights underscore the need for continued research to unravel the intricacies of genotype-performance relationships in sheep populations, thereby informing more targeted breeding and management strategies (Sawalha et al., 2007).

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Zu} + \mathbf{e}$$

Equation 1. Univariate mixed linear model (Suzuki, 2007)

$$\hat{\sigma}_e^2 = \frac{\mathbf{e^T y}}{n - \text{rank}(\mathbf{X})} = \frac{\mathbf{y^T y} - \hat{\mathbf{b}}^\mathbf{T}\mathbf{X^T y} - \hat{\mathbf{u}}^\mathbf{T}\mathbf{Z^T y}}{n - \text{rank}(\mathbf{X})}$$

$$\hat{\sigma}_u^2 = \frac{\hat{\mathbf{u}}^\mathbf{T}\mathbf{A^{-1}}\hat{\mathbf{u}} + \text{tr}(\mathbf{A^{-1}C^{22}}) \cdot \sigma_\mathbf{e}^\mathbf{2}}{\text{rank}(\mathbf{A})}$$

Equation 2. Univariate mixed linear model successive iterations (Suzuki, 2007; Mrode, 2005)

Table 1. Estimation of genetic parameters

Genetic parameters	Vp	H^2	VG_A	VG_E
Genotypes	9.5	0.69	6.6	2.9
Weight	0.97	0.17	0.17	0.80
Color	11.09	0.40	6.63	4.46

Table 2. Estimation for variance components

Genotypes	Genetic variance components			
	VGA	VGE	VP	H^2
ARR/ARR	0.8	0.4	1.2	0.33
ARR/ARQ	1.2	0.6	1.8	0.33
ARQ/ARQ	0.9	0.5	1.4	0.36
ARH/ARQ	1.5	0.8	2.3	0.35
AHQ/ARQ	1.0	0.3	1.3	0.23
ARH/ARQ	0.7	0.2	0.9	0.22
ARR/AHQ	0.5	0.1	0.6	0.17

From the data obtained in Table 1, the heritability estimated for the robe color resulted in a moderate heritability coefficient. The heritability of coat color in Karakul lambs can vary depending on the specific genetic makeup of the flock and the breeding practices employed. Generally, heritability refers to the proportion of observed variation in a trait (such as coat color) that can be attributed to genetic differences among individuals within a population. In Karakul sheep, coat color is determined by a combination of genetic factors, including genes responsible for pigmentation such as those controlling the production of melanin. The heritability of coat color in Karakul lambs is likely to be moderate to high, meaning that a significant portion of the variation in coat color among lambs is due to genetic factors. Overall, while heritability plays a significant role in determining coat color in **Karakul lambs, it is also influenced by** environmental factors and can be subject to selective breeding practices aimed at achieving desired color traits.

The tabulated genetic parameters offer valuable insights into the underlying determinants of phenotypic variation observed within the studied population. These parameters encapsulate fundamental aspects of genetic architecture and environmental influences governing trait expression.

Heritability (h^2) serves as a pivotal metric delineating the extent to which genetic factors contribute to the observed phenotypic variation. A heritability estimated of 0.69 for genotypes signifies that a modest proportion (69%) of the variability in genotype manifestation can be ascribed to genetic disparities among individuals. Conversely, traits such as weight and color exhibit comparatively lower heritability values (0.17 and 0.40, respectively), indicating a more substantial genetic influence on their phenotypic variability.

Genetic variance (VGA) delineates the magnitude of variability in a trait that can be exclusively attributed to genetic disparities among individuals. The VGA estimate of (7.2) for genotypes underscores the relatively higher contribution of genetic factors to the observed variation, whereas weight (0.17) and color (6.63) manifest greater genetic variability.

Conversely, environmental variance (VGE) quantifies the portion of phenotypic variation attributable to non-genetic factors, encompassing environmental influences and stochastic effects. A VGE estimate of (3.0) for genotypes denotes the considerable influence of environmental factors on genotype expression, while weight (0.80) and color (4.46) evince substantial environmental contributions to their phenotypic variability.

These findings underscore the complex interplay between genetic and environmental determinants in shaping trait expression. While genetic factors exert a discernible influence, environmental influences play a pivotal role in modulating phenotypic outcomes. Such

insights are pivotal for devising targeted breeding strategies and management interventions aimed at optimizing trait expression and genetic progress within the population.

Näsholm (2005) suggests that the quality attributes of Pelt in Gotland lambs exhibit moderately high heritabilities. Factors such as lamb gender, ewe age, litter size, lamb age, and their interactions exert influence on pelt quality. To enhance Pelt quality within the Gotland breed, incorporating an overall score as a trait in genetic assessments could be beneficial. The heritability estimate for the overall score is approximately 30%, with generally positive genetic correlations observed with individual pelt quality traits. Pursuing breeding strategies aimed at improving the overall score is anticipated to yield short-term enhancements in hair quality, curliness, and color nuances. However, increasing curl size and fleece thickness may not always be advantageous as these traits have optimal values. To maintain optimal levels of curl size, fleece thickness, and color nuances in the Gotland breed, strategies such as restricted selection indexes and disassortative mattings are proposed (Näsholm, 2005).

The data depicted in Table 2 offer valuable insights into the dispersion of genetic variance components among diverse genotypic classes. Grasping these components holds paramount significance in elucidating the genetic underpinnings of phenotypic diversity within sheep populations, especially concerning traits linked to scrapie susceptibility and other productive attributes.

The emREmL model has significant limitations when applied to the calculation of categorical values due to the considerable variability in genetic variance components among different genotypes. For instance, the additive genetic variance (VGA) ranges from 0.5 for ARR/AHQ to 1.5 for ARH/ARQ, while the total phenotypic variance (VP) ranges from 0.6 to 2.3. This high variability highlights that the estimated categorical values can be affected by the specificity of the genotypes and the environmental component (VGE), which also varies significantly. Additionally, it should be noted that the estimation of genetic parameters is not the primary objective of this study. The genetic parameters for genotypes were estimated for orientation purposes only, and the results should be interpreted only in the context of the number of individuals included in the study, while also considering the limitations of the linear models used.

The estimations for variance components among genotypic classes unveil noteworthy disparities in genetic variance components across distinct genotypic classes. Notably, the additive genetic variance (VGA) displays substantial variations, with elevated values evident in genotypic classes such as ARR/ARQ and ARQ/ARQ, in contrast to others like ARH/ARH and ARR/AHQ. This disparity implies differing genetic contributions to phenotypic variation among the various genotypic classes.

Environmental variance (VGE) and total phenotypic variance (VP) similarly showcase variations across genotypic classes. While environmental variance remains relatively stable across most genotypic classes, VP manifests more pronounced discrepancies, signifying the amalgamated influence of genetic and environmental factors on phenotypic manifestation. Notably, the ARQ/ARQ genotype demonstrates the highest VP, indicating substantial phenotypic variability within this genotypic class.

The identified variance components offer insights into the plausible genetic foundation of scrapie susceptibility and other productive traits. Elevated VGA in specific genotypic classes, particularly those linked with heightened susceptibility to scrapie (e.g., ARQ/ARQ), intimates a robust genetic influence on these traits. Comprehending these genetic influences is imperative for devising selective breeding schemes aimed at augmenting resistance to scrapie and enhancing overall productivity within sheep populations.

Despite the informative nature of the variance component estimates, it is imperative to acknowledge certain limitations. These estimates are contingent upon the available dataset and may be influenced by factors such as sample size, environmental variability, and genetic heterogeneity within genotypic classes. Subsequent research endeavors should prioritize the validation of these findings using larger and more diverse datasets, integrating

supplementary environmental factors, and exploring genotype-environment interactions to refine our comprehension of genetic influences on scrapie susceptibility and productive traits.

The findings accentuate the significance of integrating genetic insights, particularly pertaining to scrapie susceptibility, into breeding programs aimed at bolstering sheep health and productivity. Selective breeding strategies that prioritize individuals harboring genotypes associated with high resistance to scrapie, while concurrently considering their comprehensive genetic merit for productive traits, hold the potential to mitigate disease risks and enhance the resilience of sheep populations.

$$Y_i = b_0 + b_1X_{1i} + b_2X_{2i} + b_nX_{ni} + u_i$$

Y_i = dependent variable
b_0 = Intercept
$b_1 \ldots b_n$ = Coefficient of Regression
$X_{1i} \ldots X_{ni}$ = independent variable
u_i = disturbance error

Equation3. Multiple regression linear model (2)

Table 3. Correlation coefficients between Scrapie genotypes and lamb productive traits expressed as multiple regression

Corelation between genotypes and productive traits	Estimate	Std. Error	t value	Pr (>|t|)
Weight at birth	4.60	0.86	5.331	2.61***
ARH/ARH	-0.88	0.83	-1.063	0.28
ARH/ARQ	-0.15	0.49	-0.320	0.74
ARQ/ARQ	-0.18	0.45	-0.402	0.68
ARR/AHQ	-0.82	1.08	-0.763	0.44
ARR/ARQ	0.19	0.46	0.041	0.96
ARR/ARR	0.097	0.66	0.0146	0.88
Coat color	-0.054	0.020	-2.718	0.00714**
Lamb score	0.0005	0.0015	0.378	0.70

Data obtained in Table 3, shows that there is no correlation between resistant or susceptible genotypes and production characteristics ($p<1$). Although a weak significance is observed at the level of the coat color character ($p<0.001$), in relation to the weight of the lamb at birth.

The positive estimate of 4.60 suggests that there is a positive correlation between the weight of lambs at birth and the trait being analyzed. In other words, lambs with certain genotypes may tend to have higher birth weights compared to others.

The high t-value of 5.331 indicates that this correlation is statistically significant, meaning that it is unlikely to have occurred by random chance alone. Therefore, there is strong evidence to support the relationship between genotype and birth weight.

Each genotype (ARH/ARH, ARH/ARQ, ARQ/ARQ, etc.) has an associated estimate, standard error, t-value, and p-value.

The estimates represent the strength and direction of the correlation between each genotype and the productive traits. A negative estimate suggests a negative correlation, while a positive estimate indicates a positive correlation. The t-values measure the significance of the correlation. Higher absolute t-values indicate stronger evidence against the null hypothesis of no correlation. The p-values indicate the probability of observing the correlation if there were no true association between the genotype and the trait. Lower p-values suggest stronger evidence against the null hypothesis.

The negative estimate of -0.054 suggests a negative correlation between coat color and the trait being analyzed. This implies that lambs with certain coat colors may tend to have lower values for the trait. The significant t-value of -2.718 indicates that this correlation is statistically significant, providing strong evidence against the null hypothesis of no correlation.

The estimate of 0.0005 suggests a very weak positive correlation between lamb score and the trait being analyzed. This indicates that there may be a slight tendency for lambs with higher scores to exhibit higher values for the trait, although the correlation is very weak.

The non-significant t-value of 0.378 suggests that this correlation is not statistically significant, meaning that there is insufficient evidence to conclude that the relationship between lamb score and the trait is not likely due to random chance.

CONCLUSIONS

Despite our efforts to comprehensively analyse the relationship between PRP gene polymorphisms and various traits, it's essential to acknowledge the potential limitations of our dataset used in this paper. Factors such as

sample size, genetic diversity within the population, and environmental influences may have impacted the robustness of our findings. Future studies with larger and more diverse datasets could provide deeper insights into these relationships.

Our study primarily focused on specific traits such as birth weight, coat color, and scrapie resistance, leaving out other potentially relevant factors that could influence sheep productivity. Factors such as nutrition, management practices, and environmental conditions were not explicitly accounted for in our analysis. Incorporating these additional variables into future research endeavors could offer a more holistic understanding of the genetic and environmental determinants of sheep performance.

Previous research endeavors have explored the relationship between PRP gene genotypes and various production characteristics, including subcutaneous fat percentage, muscle mass, and milk physicochemical parameters. Although some studies have hinted at potential interactions, the significance levels of these associations remain uncertain, leading to conjecture regarding the possibility of false-positive findings.

While our analysis did not uncover direct correlations between resistant genotypes and productive traits, it is worth noting the potential for individualized selection strategies to enhance scrapie resistance. However, any such selection processes must carefully consider the broader context of individual animals' productive performances.

It is imperative to recognize the intricate interplay of numerous variables influencing the productive performance of sheep populations. Further investigations are warranted to gain a more comprehensive understanding of these complex relationships and their implications for breeding and management practices.

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