Analysis of scrapie resistance and productivity of Latvian dark head breed old type sheep compliant to the conservation program of the genetic resources

D. Bārzdiņa and D. Kairiša

Institute of Animal science, Faculty of Agriculture, Latvia University of Life Sciences and Technologies, Lielā iela 2, Jelgava, LV–3001, Latvia
*Correspondence: dace.barzdina@lbtu.lv, daina.kairisa@lbtu.lv

Abstract. Since 2002, the European Union has implemented a disease surveillance programme for transmissible spongiform encephalopathy (TSE) in small ruminants, which can cause very high economic losses to sheep farmers worldwide. In order to detect susceptibility to this fatal sheep disease, surveillance of this disease has been carried out in Europe for several years. Such a programme was introduced in Latvia in 2004 when the country joined the European Union, so Latvia has been monitoring this potential disease for several years. As this fatal disease has not been detected in Latvia, only a few studies have been conducted on scrapie genotypes in the Latvian Dark Head sheep breed and their effect on sheep productivity traits. The aim of the study was to analyse the productivity of the first lambs of Latvian Dark Head sheep in relation to the scrapie genotype. According to the data analysis, a total of 1531 or 97.7% of the ewes with R1, R2 and R3 scrapie genotypes can be used for future sheep breeding. The average age of all ewes included in the Programme was 5 years and the average fertility was 1.8 lambs. Later in the work, 1,335 Latvian Dark Head ewes with 100% bloodiness were analysed and the average fertility of ewes in the R1 and R3 scrapie genotype groups was 1.6 lambs and 1.7 lambs for ewes in the R2 risk group. In the last 10 years, the implementation of the conservation programme of the old type Latvian Dark Head sheep breed has been successful.

Key words: genotype, scrapie resistance, genetics resources, ewe's productivity, first lambing.

INTRODUCTION

At the centre of the European Union's Green Deal strategy for sustainable and inclusive growth is the health of society as a whole, safeguarding nature's diversity and the quality of the environment. Its aim is to stimulate economic growth through environmentally friendly solutions, without having a negative impact on nature and its resources. One of the components of the European Green Deal is to reduce the rate of extinction of animal species (A European Green Deal, 2019). In 2014, the proportion of livestock breeds classified as threatened with extinction worldwide increased by 17%. A proportion of breeds (58%) are classified as having an unknown risk status due to a lack of recent population data. Reduced diversity of native breeds as a result of inbreeding may be an issue, even for breeds whose overall population size remains large

(Scherf & Pilling, 2015). Transmissible spongiform encephalopathy (TSE), or scrapie disease, is found almost worldwide in sheep and goats and has been known for about 250–270 years. It is a prion disease, a fatal neurodegenerative disease caused by conformational changes in the cellular prion protein (PrPC) of the pathogenic form (PrPSc). There are two forms of scrapie: classical and atypical. Susceptibility or resistance of sheep to classical scrapie is tightly controlled by polymorphisms in PRNP codons 136, 154 and 171. A genetic role in sheep atypical scrapie has been determined by polymorphisms in codons 141, 154 and 171. Although classical scrapie is clearly a naturally occurring contagious disease, atypical scrapie is probably not contagious and is caused by agerelated spontaneous misfolding of the prion protein (Simmons et al., 2009; Acín et al., 2021; Fast & Groschup, 2023). In general, sheep breeding plans aim to reduce susceptibility alleles in classical scrapie (VLRQ) and atypical scrapie (AFRQ, ALRH) cases and increase the resistance allele ALRR (Grāve & Granta, 2012).

In Latvia, this fatal disease has not been detected, therefore few studies have been carried out on the scrapie genotypes of the Latvian Dark Head sheep breed and their effect on the productivity traits of ewes. Sheep of the Latvian Dark Head breed are bred in Latvia, which are used for the implementation of the programme 'Preservation of genetic resources of agricultural animals' of local breeds. Sheep of the local breed are characterised by a strong body structure and are able to pass on their vitality to their offspring. The Latvian Dark Head breed can serve as a genetic reserve to ensure the diversity of the sheep population. We consider the problem of the study to be the reduction of gene diversity in the population, which could lead to a decrease in the productivity of ewes.

The aim of the study is to analyse the first lambing productivity of ewes of the Latvian Dark Head old type breed in relation to the scrapie genotype.

MATERIALS AND METHODS

The Latvian Dark Head sheep breed conservation programme (forward Programme) is being implemented in Latvia, where in the study included ewes that appropriate the requirements of the conservation programme (Agricultural data centre, 2019). In order to achieve the goal of the Programme, one of the points is to use scrapie resistant sheep for breeding. The implementation of the Programme started in 2004. The Latvian Dark Head old type breeding Programme is implemented by the 'Latvian Sheep Breeders' Association'. Since 2012, the Society has been cooperating with the German partner laboratory EUROFINS and since 2017 with the Institute for Food Safety, Animal Health and Environment 'BIOR' where blood samples were collected and analysed according to generally accepted standards and methods who are described previously in our study (Barzdina & Kairisa, 2021). The ewes included in the study are kept on breeding farms, which must be followed the welfare rules established in the Republic of Latvia (General Welfare Requirements…, 2008). The study analysed different scrapie genotypes of the animals included in the Programme. These included both classical and atypical scrapie genotypes. The most resistant animals to classical and atypical scrapie that can be used for population reproduction are assigned to the haplogenic combination in the ALRR risk group R1. In Latvia animals assigned to the haplogene combination VLRQ and AFRQ risk groups R4 and R5 shall not be used for population breeding (Table 1). From 2004 to 2020, the productivity parameters of first lambing ewes were

analysed. In total, the parameters of 1,567 ewes were analysed, divided into the five scrapie risk groups. In Latvia, when the conservation programme started in 2004, it was allowed to receive animals crossed with rams of related breeds (German black-faced breed).

Table 1. The description of ewe's scrapie genotype and risk groups included in the study

Genotype (136/141/154/171)	Risk group	The use of sheep breeding
Classical scrapie (CS)		
ALRR/ALRR	R1	Most resistant and are used for breeding
ALRR/ALHQ	R2	Resistant and are used for breeding
ALRR/ALRH		
ALRR/ALRQ		
ALRQ/ALRQ	R3	Reduced resistance to scrapie
ALRQ/ALHQ		Used for breeding in small populations cases
ALRQ/ALRH		
ALRH/ALRH		
ALRR/VLRQ	R4	Order to preserve rare breeds permissible to use reproducing at the individual parental mating
ALRQ/VLRQ	R5	Very susceptible to scrapie. Do not use for selection and breeding
Atypical scrapie (AS)		
ALRR/AFRQ	R5	Very susceptible to scrapie. Do not use for selection and breeding
ALRQ/AFRQ		
ALRH/AFRQ		

As the proportion of purebred animals increases, only 100% purebred animals are accepted into the Programme. In order to determine how many purebred (LT100%) and crossbred (LT75%) ewes were included in the Programme, they were divided into sheep groups and the percentage was calculated (Table 2).

The highest percentage of sheep in both the LT100% (42.1%) and LT75% (40.9%) groups were assigned to the R2 scrapie risk group according to the scrapie analyses performed. The most resistant group included sheep in the LT100% group, 36.9%, and only 14.4% in the LT75% group. This can be explained by the decision of the owners to breed purebred sheep with resistant genotypes in the future. Only sheep with the most resistant (R1), resistant (R2) and reduced (R3) risk groups against scrapie and only purebred (LT100%) ewes, which may be used for selection and breeding in Latvia, were included for further data analysis. Therefore, the frequency of ewes (LT100%), Skrepi genotype depending on the year of birth of the ewes can be seen in Table 3. Since 2004, when this conservation Programme was started, there has been a positive trend in the increase of animals of the desired scrapie genotype (R1, R2 and R3).

Table 2. Number of animals included in the study and percentage distribution by risk groups depending on the sheep groups

Risk groups	Sheep groups				Total for the risk groups
	LT100%		LT75%		
	n	%	n	%	
R1	502	36.9	30	14.4	532
R2	572	42.1	85	40.9	657
R3	261	19.2	81	38.9	342
R4	3	0.2	2	1.0	5
R5	21	1.6	10	4.8	31
Total	1,359	100.0	208	100.0	1,567

As in our previous study on the frequencies of scrapie genotypes in ewes from 2004 to 2012 (Bārzdiņa & Kairiša, 2014), the number of ewes of the preferred scrapie genotypes is increasing in this study. The most resistant (R1) scrapie genotype has increased by 34.2% since 2013, where the undesirable (R5) scrapie genotype ewes have not been included in the Programme since 2017.

The collected data were analysed using mathematical data processing methods and the mean and standard deviation of the traits were determined. To compare the interaction of different factors was used two - way ANOVA without replication. Significance between differences in means was determined by Student's *t*-test. Significant differences between risk groups were marked with small Latin letters: a and b, and between sexes - with capital Latin letters A and B ($p \leq 0.05$).

Table 3. Scrapie risk group distribution of Latvian Dark Head ewes' birth year, %

Birth years of sheep	The risk groups of scrapie				Total number of ewes per year of birth
	R1	R2	R3	R5	
2013	30.3	41.5	24.6	3.5	142
2014	38.5	45.2	14.8	1.5	135
2015	39.9	46.9	12.6	0.7	143
2016	41.3	41.3	16.5	0.9	109
2017	43.9	43.9	12.1	0.0	132
2018	50.6	40.2	9.2	0.0	87
2019	55.7	35.4	8.9	0.0	79
2020	64.5	32.3	3.2	0.0	93

RESULTS AND DISCUSSION

All first lambing ewes included in the study were genotyped regarding for PrP codons 136, 141, 154 and 171 and divided into scrapie risk groups (Table 4).

According to the data collected, 34.5% of the first lambing ewes were in the most resistant group with the ALRR/ALRR genotype, but 43.1% of the animals were in the R2 risk group. It shows that, thanks to careful animal selection, the number of animals with the resistant scrapie genotype is increasing in the population of genetic resources of the Latvian Dark Head breed. Most of the ewes showed resistant genotypes (ALRR, ALHQ, ALRH, ALRQ) 43.1%, which were classified as R2 scrapie risk group. In the study conducted in the United Kingdom (UK) in 2016 with British sheep breeds, 35.7% of the animals were classified as R2 risk group (Yaman & Ün, 2017).

Table 4. Genotype frequencies of scrapie in the Latvian Dark Head breed ewe's, %

Genotype (136/141/154/171)	Risk group	Frequency, % $n = 1,335$
ALRR/ALRR	R1	34.5
ALRR/ALHQ	R2	0.6
ALRR/ALRH		2.8
ALRR/ALRQ		39.7
ALRH/ALRH	R3	0.1
ALRH/ALRQ		2.0
ALRQ/ALHQ		0.7
ALRQ/ALRQ		19.7

Early coupling of sheep can lead to body defects and health problems, but late coupling of sheep has an impact on the farm's part of lost production. On farms the owners' sheep start mating at an average age of 1.5 to 2 years.

When analysing the variance with ANOVA tools, the ewes age of first lambing is influenced by factors such as the year of birth of the ewe and the scrapie genotype, and the farm factor does not influence this indicator. Also, when analysing number of lambs born per ewe and lamb's liveability, none of mentioned factors influence these parameters. The productivity parameters of the first lambing of purebred ewes are shown in Table 5.

Table 5. First lambing purebred ewe's productivity parameters depending on scrapie risk groups

Risk groups	Ewes age of first lambing, days	Number of lambs born per ewe	Lamb's liveability, %	Live weight of lambs at the age of 70 days, kg	
				female	male
	$\bar{x} \pm S\bar{x}$				
R1	653.0 ± 6.15[a]	1.6 ± 0.03[a]	97.3 ± 0.58[a]	20.8 ± 0.23[aA]	21.9 ± 0.26[aB]
R2	702.6 ± 6.43[b]	1.7 ± 0.02[a]	97.1 ± 0.53[a]	20.2 ± 0.22[bA]	21.0 ± 0.24[bB]
R3	722. 0 ± 8.66[b]	1.6 ± 0.04[a]	94.7 ± 1.18[a]	19.7 ± 0.32[bA]	20.8 ± 0.46[bB]

[a, b, A, B] – observed significant differences, $p \leq 0.05$.

Purebred (LT100%) ewes with the most resistant genotype (R1) were mated faster at 653.0 ± 6.15 days. Significant differences in the age at first lambing were observed in the ewes with the R1 scrapie genotype. This shows that the requirements of the Programme are being followed on the farms and that the owners want to breed animals with resistant genotypes as soon as possible. The average number of lambs born per ewe and lamb liveability was not significantly different and ranged from 1.6 to 1.7 lambs and 97.3 to 94.7% survival. Significant differences in lamb live weight at 70 days of age were observed between risk groups and lamb sex. In a study conducted in Poland with local sheep breeds, the number of lambs born per ewe in risk group R1 was 1.8 ± 0.05 lambs with an average live weight at 56 days of age of 19.8 ± 0.61 kg (Gruszecki et al., 2012). The selection of ewes for breeding with scrapie resistance genotypes (R1 and R2) has a positive effect on animal productivity.

When analysed by ANOVA tools live weight of lambs at 70 days and live weight gain per day, factors such as lambing year, scrapie genotype and farm had no significant interaction. However, there was an interaction between sexes for live weight and daily gain. The daily live weight gain of the offspring of the ewes included in the study by scrapie risk groups is shown in Fig. 1.
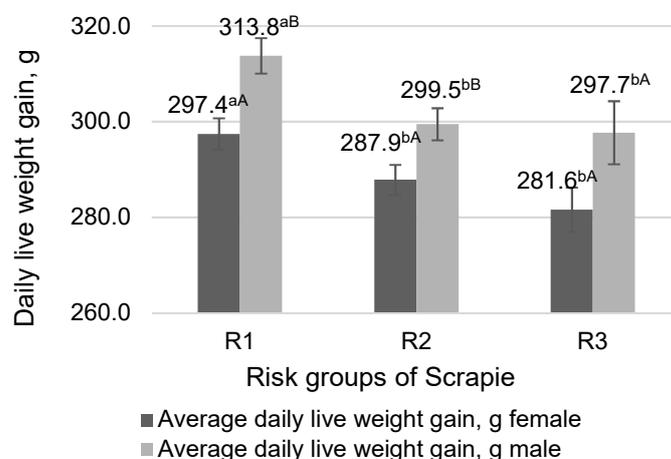
Figure 1. Average daily live weight gain of offspring depending on sex by risk and sheep groups.
[a, b, A, B] – observed significant differences, $p \leq 0.05$.

When analysing the daily live weight gain of the offspring of the research sheep, animals included in the R1 risk group (297.4 ± 3.31 and 313.8 ± 3.70 g) showed a

significant difference between the R2 and R3 offspring groups. When analysing the data on daily live weight gain between the sexes of the offspring of ewes, significant differences were observed between the animals included in the R1 and R2 risk groups.

In a study carried out in Hungary in 2009 with Hungarian and German meat merino sheep with different PrP genotypes, the highest daily live weight gain was observed in the German meat merino offspring - 383.14 kg (Nagy et al., 2009).

CONCLUSIONS

During the last 10 years, the implementation of the conservation Programme of the old type Latvian Dark Head sheep breed has been successful. Ewes with the R1 and R2 scrapie genotypes show better productivity parameters. There is a growing interest in using purebred R1 and R2 genotype rams for mating ewes, which results in genetically homogeneous flocks.

REFERENCES

A European Green Deal 2019. *https://commission.europa.eu/strategy-and-policy/priorities-2019-2024/european-green-deal en.* Accessed 25.01.2023.

Acín, C., Bolea, R., Monzón, M., Monleón, E., Moreno, B., Filali, H. & Badiola, J.J. 2021. Classical and atypical Scrapie in sheep and goats. Review on the etiology, genetic factors, pathogenesis, diagnosis, and control measures of both diseases. *Animals* **11**(3), 2–20.

Agricultural data center 2019. Latvian Dark Head old type sheep breed conservation program. Available at https://www.ldc.gov.lv/lv/media/980/download?attachment (in Latvian)

Bārzdiņa, D. & Kairiša, D. 2014. Analysis of Scrapie genotype of Latvian Darkhead sheep genetic resources. *Gyvulininkystė: Mokslo Darbai.* **62**, 62–72.

Barzdina, D. & Kairisa, D. 2021. The Analysis of Scrapie Genotype of the Genetic Resources of Latvian Dark Head Breed. *International Multidisciplinary Scientific GeoConference: SGEM* **21**(6.1), 131–138.

Fast, C. & Groschup, M.H. 2023. Classical and atypical Scrapie in sheep and goats. *Prions and Diseases* **2**, 533–563.

General Welfare Requirements for Farm Animals 2008. Legal Acts of The Republic of Latvia. Available at https://likumi.lv/ta/en/en/id/168773 (in Latvian).

Grāve, K. & Granta, R. 2012. PRNP genotype prevalence in Latvian darkheaded sheep breed. *Dzīvnieki. Veselība. Pārtikas higiēna. Animals. Health. Food Hygiene*, 40–45.

Gruszecki, T.M., Greguła-Kania, M., Niżnikowski, R., Pięta, M., Kostro, K., Szymanowska, A., & Strzelec, E. 2012. Effect of PRNP gene polymorphism on reproductive performance of mother sheep and their offspring growth. *Journal of Veterinary Research* **56**(3), 279–282.

Nagy, B., Anton, I., Safar, L., Fésüs, L. & Zsolnai, A. 2009. Association between PrP genotypes and selected growth traits of Hungarian Merino and German Mutton Merino rams. *Archives Animal Breeding* **52**(6), 613–617.

Scherf, B.D. & Pilling, D. 2015. The second report on the state of the world's animal genetic resources for food and agriculture. *Food and Agriculture Organization of the United Nations*, 604 pp.

Simmons, H.A., Simmons, M.M., Spencer, Y.I., Chaplin, M.J., Povey, G., Davis, A. & Wrathall, A.E. 2009. Atypical Scrapie in sheep from a UK research flock which is free from classical Scrapie. *BMC veterinary research* **5**(1), 1–9.

Yaman, Y. & Ün, C. 2017. Genetic Resistance to Prion Diseases. **In** Prion-An Overview. IntechOpen, *https://www.intechopen.com/chapters/54192.* Accessed 25.01.2023.