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Original article

PrP GENE POLYMORPHISM AND ITS INFLUENCE ON SOME PRODUCTIVE TRAITS OF SHEEP BREEDS REARED IN BULGARIA

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Summary

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The rapid dissemination of scrapie over the past few decades led to development of a specific eradication programme, based on the polymorphisms within the prion protein gene (*PRNP*). Current approach encourages the selection of animals carrying the resistant ARR/ARR genotype, while other genotypes are considered less preferable. Although the strategy seems to be working quite well, farmers are concerned whether this will affect sheep productivity and subsequently decrease net profits. The current study was aimed to elucidate the linkage between the *PrP* gene polymorphism (based on codons 136, 154 and 171) and some productive traits (live weight, reproduction, milk and fleece yield) of three sheep breeds reared in Bulgaria – Assaf, Northeast Bulgarian Merino and Blackhead Pleven. The total number of detected genotypes was six – ARR/ARR, ARR/ARQ, ARR/ARH, ARQ/ARQ, AHQ/ARQ and ARR/VRQ, with different prevalence within each breed. The observed lack of significant differences in the studied performance traits between the *PRNP* genotypes suggests that *PRNP* polymorphisms did not influence the sheep productive performance. Therefore, selection of animals on the resistant genotype (ARR/ARR) would not worsen their productivity. The obtained results should help the better understanding of scrapie selection and the positive effect that it would have to both health care and industry.

Key words: PCR-RFLP prion, *PRNP*, productivity, scrapie, sheep

INTRODUCTION

For the past few centuries, both science and practice have dramatically improved the production traits among all domestic animals, which boosted the food production responding to the constantly growing population on Earth. Sheep are used for production of meat, milk, leather and wool, and all four products are highly used in many industries. Nowadays genetic tools allow the enhancement of animals' productivity via genotype-based breeding.

Scrapie is a neurodegenerative disease affecting sheep and goats, characterised by long incubation period and relatively high mortality rates. The disease is caused

by an infectious protein known as prion. The first cases have been described more than 280 years ago (1732), in Britain (Plummer, 1946). Scrapie belongs to a group of similar diseases known as transmissible spongiform encephalopathies (TSE) affecting the central nervous system of both animals and humans (Prusiner, 1998). The clinical appearance of the disease is in strong relation with the polymorphic *PRNP* gene, localised in chromosome 13 of sheep's genome (Konold *et al.*, 2010). So far polymorphisms have been detected in 29 codons, and three of them – 136, 154 and 171 are considered as highly associated to the classical disease (Piestrzyńska-Kajtoch *et al.*, 2011; Koynarski & Hristova, 2015). Depending on the nucleotide sequence in these three codons, the final protein has variable amino acid composition – codon 136 could encode alanine or valine (A136V), codon 154 could outline histidine or arginine (H154R) and codon 171 can determine histidine, glutamine or arginine (H171QR). All combinations among these codons are still not discovered, but the commonest are VRQ, ARH, AHQ, ARQ and ARR. According to the classification of Ulvund (2006), these five haplotypes and the genotypes formed by them belong to different scrapie risk groups. At present, most authors share the vision that the ARR/ARR is the most resistant genotype, which corresponds to the classification of this genotype as "highly resistant" in the National scrapie plan of Great Britain (group 1) (Baylis *et al.*, 2004; Goldmann *et al.*, 2006). Contrary, genotypes AHQ/VRQ, ARH/VRQ, ARQ/VRQ, VRQ/VRQ belong to group 5 (highly susceptible) (DEFRA, 2001). Due to its unconventional appearance, the scrapie disease is very difficult to be diagnosed and managed and requires clear determination of sheep genotype and expected susceptibility.

Current scrapie eradication programme includes genotyping and subsequent selection of animals on the ARR/ARR genotype. This widely accepted approach, motivated German researchers to investigate the relation between the *PrP* gene polymorphism and some production traits (muscle mass, wool quality, milk yields, age at first lambing, first lambing interval, second lambing interval etc.) among seven sheep breeds. Authors did not obtain any statistically significant data in favour of any of the observed genotypes. These results confirm the idea that this gene has effect on susceptibility to the disease, but no on animals' production traits (De Vries *et al.*, 2004). A similar experiment was conducted by Matthews (2015). The author has reported better lifetime breeding success of animals homozygous for the ARR haplotype, compared with carriers of any other genotype. Interestingly, the investigated animals did not suffer from either pre-clinical or clinical scrapie, which explains the better results in favour of the ARR/ARR genotype.

The present study aimed to elucidate the linkage between different *PRNP* genotypes and some production traits (live weight, fertility, milk and wool yield) in three sheep breeds, reared in Bulgaria.

MATERIALS AND METHODS

Experimental animals

The *PRNP* genotypes were analysed in ewes from Assaf, Northeast Bulgarian Merino and Blackhead Pleven breeds. All experimental animals were between 2 and 3 years of age and farmed under semi-intensive technology. Blood samples for

analysis (100 samples from each breed; total number of samples 300) were collected aseptically from *v. jugulars* with disposable needles in plain vacutainers after restraint. Blood was transported in cool bags at 6–7 ºC.

Genotype detection

DNA was extracted from peripheral blood using QIAamp DNA Mini Kit (Qiagen).

PrP genotypes were determined by the PCR-RFLP method of Lühken *et al.* (2004). To distinguish between seven *PrP* haplotypes based on polymorphisms at codons 136,154 and 171, two different PCR fragments (197 bp and 196 bp) were amplified in separate PCR reactions. The reaction mixture was with final volume of 25 µL and comprised approximately 50 ng of genomic DNA, 20 pmol of each primer, 5 mM of each dNTP, 2.0 mM (197 bp fragment) or 1.5 mM (196 bp fragment) $MgCl_2$ and 0.1U Taq polymerase in 1-fold reaction buffer. After initial denaturation at 95 °C for 5 min, 36 amplification cycles were performed, including denaturation at 95 °C for 30 s, annealing at 56 °C (for both reactions) for 40 s and extension at 72 °C for 1 min, followed by a final 5-min extension at 72 °C. Both expected fragments were amplified by the same forward primer 5'-TGTGGCAGGAGCTGCTGC AGCT-3', which spans nucleotides 22619 to 22640 of GenBank sequence U67922. The 197 bp fragment was produced in PCR with a modified reverse-primer 5'-TGCACAAAGTTGTTCTGGTTACT ATC-3', which spans nucleotides 22791 to 22816 of GenBank sequence U67922. This PCR fragment contained an artificial restriction site for *Bsp*HI, when the codon for histidine occured at position 171. The other reverse primer 5'-GCACAAAGT TGTTCTGGTTACTATAT-3', which is a nucleotide 22790 to 22815 of GenBank sequence U67922, amplified the 196 bp fragment with an artificial restriction site for *Bsp*DI in the case of the codon for arginine at position 171. In both fragments, the codon for valine at position 136 and for histidine at position 154 formed restriction sites for *Bsp*HI. The 197 bp fragment was digested with *Bsp*HI, while the 196 bp fragment was double digested with *Bsp*HI and *Bsp*DI simultaneously; samples were incubated for 4 h at 37 °C. Electrophoresis was done in a 3.5% agarose gel (1:1 mixture of low melting agarose and standard agarose) at 7.5 V/cm in 1× TBE buffer at 2 °C. The products were visualised after ethidium bromide staining under UV light (Fig. 1).

Productive parameters measurement

The body weight (kg) and fertility (lambs per year) were examined for all three breeds. Milk productivity (milk yield per lactation – kg) was determined for the Blackhead Pleven breed and wool production (fleece yield per year – kg and fibre length – cm) was analysed for the Northeast Bulgarian Merino breed.

Statistical analysis

Obtained data were statistically processed by one-way analysis of variance (ANOVA) with fixed effects of the factor using the Microsoft Excel Data Analysis tool pack 2016.

RESULTS

Table 1 presents the linkage between the *PRNP* genotypes and the productive parameters of interest for all sheep breeds. The influence of the *PRNP* haplotypes on live weight and fertility rate of the animals were explored among all three breeds of interest. As seen from the table, the highest body weight was found among the

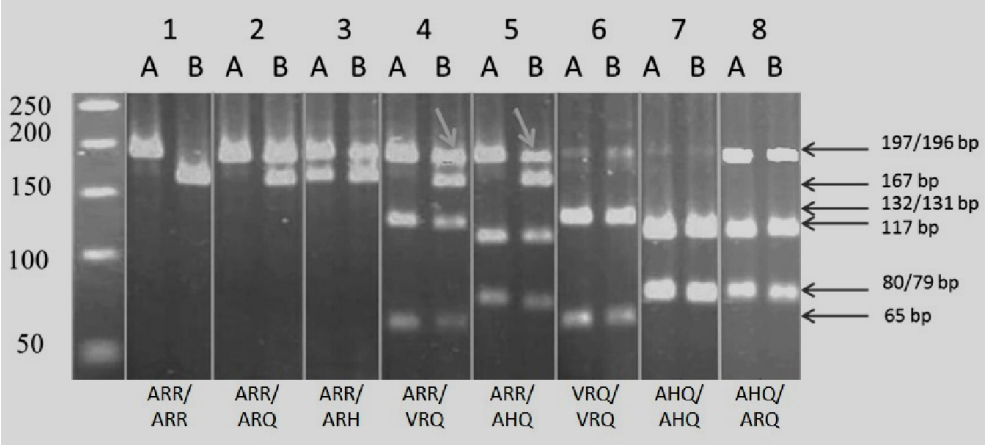

Fig. 1. *PRNP* genotyping using the PCR-RFLP method of Lühken *et al.* (2004). Different genotypes are presented in lanes 1–8 separated by agarose gel electrophoresis and stained with ethidium bromide. A: PCR fragment 197 bp digested with the restriction enzyme *Bsp*HI; B: PCR fragment 196 bp simultaneously digested with *Bsp*HI and *Bsp*DI. Post digestion fragment sizes are shown with arrows. The digestion of B fragment for ARR/VRQ and ARR/AHQ genotypes leads to additional 196 bp fragment. This needs further clarification and the observed fragment is without diagnostic relevance.

carriers of the resistant genotype (ARR/ ARR) within the Assaf breed (79.15 kg), while the lowest body weight (61.58 kg) was established for the carriers of the ARR/VRQ genotype for the Blackhead Pleven breed. The results for the fertility rate were even more heterogeneous. The wild type genotype (ARQ/ARQ) exhibited the highest result within the Assaf breed (1.47 lambs per year), while the same genotype showed one of the lowest results (1.29) within the Northeast Bulgarian Merino breed.

The other two association studies targeted the relation between the scrapie-resistant genotypes and milk or wool production, respectively. Due to the breed specificity of these parameters, the influence of the *PrP* polymorphism on milk yield was analysed for the Blackhead Pleven breed, while the wool performance (fleece yield and fibre length) – for the Northeast Bulgarian Merino breed. All genotypes exhibited relatively equal results for the milk production with the exception of the AHQ/ARQ, which showed the lowest result – 114.17 kg per year. Although the resistant genotype (ARR/ARR) exhibited the highest fleece yield (6.04 kg), the fibre length was rather modest (9.04 cm). The results for all productive traits were juxtaposed to the obtained *PrP* genotypes, but significant results were not observed (P>0.05).

DISCUSSION

Within the EU, the scrapie management is designated by the EU Directive 999/2001. Additionally, the EU Commission in the 2003/100/EU decision lists the conditions and rules for implementation of breeding programmes aiming at eradication of the disease. These documents endorse selection of animals homozygous for the ARR

Table 1. Influence of different *PrP* genotypes to sheep productive traits (mean±SD; n=100)

Genotype	Risk group	n	Live weight (kg)	Fertility (lambs/year)	Milk yield (kg/year)	Fleece yield (kg)	Fibre length (cm)
Assaf							
ARR/ARR	1	77	79.15±1.92	1.46±0.21			
ARQ/ARQ	3	23	77.84±3.09	1.47±0.32			
Northeast Bulgarian Merino							
ARR/ARR	1	12	73.04±3.97	1.31±0.14		6.04±0.39	9.04±0.70
ARR/ARQ	2	30	68.92±2.89	1.37±0.23		5.81±0.35	9.10±0.54
ARR/ARH		8	65.81±6.03	1.44±0.18		5.92±0.37	9.21±0.32
ARQ/ARQ	3	18	74.12±4.88	1.29±0.15		5.68±0.22	9.07±0.12
AHQ/ARQ		10	66.83±4.14	1.10±0.17		5.29±0.38	9.04±0.48
ARR/VRQ	4	22	64.78±2.86	1.40±0.31		5.41±0.30	8.97±0.62
Blackhead Pleven							
ARR/ARR	1	15	73.59±9.51	1.64±0.22	129.41±18.32		
ARR/ARQ	2	30	71.14±4.63	1.44±0.20	131.55±14.68		
ARQ/ARQ	3	42	64.19±5.02	1.39±0.18	131.05±12.65		
AHQ/ARQ		8	67.18±6.07	1.36±0.25	114.17±12.08		
ARR/VRQ	4	5	61.58±5.92	1.37±0.12	132.19±27.06		

No significant results were obtained for any of the observed genotypes and productive parameters within each breed (P>0.05). Parameter variations between breeds are result of different breeding strategies and production types.

haplotype, which instantly raises the question of how this selection would affect sheep production traits.

The absence of significant differences between the different *PrP* genotypes with respect to the productive parameters in all investigated breeds indicates that the polymorphisms in *PRNP* gene did not affect animals' performance. The evident between-breed differences result from different breeding strategies and production types. Putting this aside, the *PRNP* polymorphism seemed to not interfere with the overall results of the livestock. The Assaf sheep breed is a popular meat/milk type breed, which corresponds to an average body weight of 80 kg. The Northeast Bulgarian Merino and the Blackhead Pleven breeds are wool and milk type breeds, which explains the moderate live weight of 65 kg. De Vries *et al.* (2004) investigated the impact of the resistant ARR/ARR genotype on the body weight, fleece yield, quality of the obtained wool and daily weight gain, among the German black-headed, German white-headed mutton, Bleu du Maine, German mutton merino, Leine, Texel and Suffolk breeds. Authors did not detect any significant differences in all production traits of interest favouring any genotype. Bearing in mind these results and combining them with our data, we could confirm that the resistant genotype of the *PRNP* was is not

associated with a negative effect on meat production.

The fertility rate is one of the most important factors in sheep industry. The observed rates in this study are within the average values for each breed. Similarly to the other productive traits, data comparing different genotypes and fertility of their carriers within each breed, demonstrated no influence of the *PrP* polymorphism on this parameter (P>0.05). A similar experiment was conducted in Spain (Casellas *et al.*, 2007). In addition to the fertility rate, authors observed the live weights of the newborn lambs. The experiment was carried out on 310 Ripollesa sheep. Although the authors investigated a larger number of animals, significant differences among the *PrP* genotypes were not found out. The Assaf breed in our investigation was imported from Germany, whereas the other two are results of crossbreeding of some local Bulgarian breeds. Taking into account our data and the results reported by Casellas *et al.* (2007) for the Ripollesa breed, we could assume that these are highly divergent breeds with totally different pedigrees. Despite this diversity, the analysis of data for the *PrP* genotypes allowed concluding that the gene polymorphism did not influence the fertility rates of the animals and that selection on the ARR/ARR genotype will not impair this parameter.

In addition to the previous two parameters, dairy sheep industry is concerned about the effect of the recommended *PRNP* selection on the milk yield and milk quality. Bulgaria has traditions in dairy sheep farming, which motivated us to investigate the influence of different *PrP* genotypes on the milk yield among the Blackhead Pleven breed. Salaris *et al.* (2007) examined the *PRNP* impact on milk productivity among 650 animals from the East Friesian breed. Despite the large number of samples, authors reported no influence for any of the detected *PRNP* gene variants to sheep's milk productiveness. Similar results were stated by Carta *et al.* (2009) having studied the impact of *PrP* polymorphism on the milk yield and quality in the Sardinian sheep breed. Authors did not obtain any relevant results for both parameters of interest. Summing up the results for the East Frisian, Sardinian breed and the Blackhead Pleven breed from our study, we could expect that selection of animals on the resistant ARR/ARR genotype would not interfere with sheep's milk production.

Wool productivity of sheep is one of the main branches of the sheep industry, which encouraged the selection of dedicated breeds with high wool quality and yields. This study aimed to clarify the impact of the *PrP* genotypes on wool production traints (fleece yield per year – kg and fibre length – cm) among the Northeast Bulgarian Merino breed. For the 100 observed animals 6 genotypes were detected, each belonging to a different scrapie risk group. As shown in the table, significant differences between the observed genotypes were not obtained neither for the fleece yield nor for the fibre length. German authors (De Vries *et al.*, 2015) conducted a similar study among seven breeds and compared the fleece yield and fibre length in sheep carriers of the resistant ARR/ARR genotype with animals positive for any other *PrP* genotype. Significant *PRNP* influence among the seven studied breeds was not obtained for neither of the two parameters. The absence of significance and the close results between different genotypes from our study and the results reported by De Vries *et al.* (2015), unambiguously illus-

trates that the *PrP* genotypes had no adverse effect on wool production in sheep.

The lack of significant relationship between all four productive traits and any *PrP* genotype, indicated the absence of influence of the polymorphic *PRNP* gene on sheep's productivity, i.e. the selection of animals on the resistant genotype (ARR/ARR) will not have a negative effect on productive parameters. These results should reduce farmers' concerns about scrapie selection and help understanding the positive effect that it would have to both health care and industry.

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