RESEARCH ARTICLE

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Genetic diversity of Booroola gene in Northeast Bulgarian Merino sheep breed

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ABSTRACT
The *BMPR-1B/FecB* or Booroola gene is the first major fecundity gene identified in sheep. The present investigation was carried out to study polymorphism by PCR-RFLP of *FecB* gene in 30 ewes belonging to the Northeast Bulgarian Merino sheep breed. Genomic DNA was extracted from whole blood. PCR amplification of *FecB* gene revealed fragments with length 190 bp using a specific primer set. After PCR-RFLP analysis it was found the wild allele "+" and the mutant allele "*B*" with frequencies 0.97 and 0.03, respectively. This is the first study in Bulgarian sheep breeds, where was found the presence of Booroola mutation. According to the results the genotype frequencies were as followed: wild homozygous genotype "++" 0.93 and heterozygous genotype "*B*+" 0.07, respectively. The observed (H_o) and expected heterozygosity (H_e) were 0.066 and 0.058, respectively. It can be concluded that the studied herd was in Hardy-Weinberg equilibrium. PCR-RFLP technique can be used to detect and genotype the *FecB* gene clearly.

Key words: Sheep, Northeast Bulgarian Merino breed, BMPR-1B/FecB gene, PCR-RFLP analysis

Introduction

In the last decade there is not only the extinction of sheep breeds in Bulgaria but also the serious fall of the number of individuals. The loss of genetic recourses is irreversible. The conservation of fine fleece sheep breeds in Bulgaria is highly decreased due to the greatly reduced interest in wool. The adequate implementation of breeding strategies is extremely necessary for preventing future damages (Stancheva et al., 2017). Applying modern DNA technologies to conventional breeding programs would contribute to faster and more single-minded production.

Improvement of reproductive traits in sheep could lead to higher litter size and therefore to increasing the profit as the litter size in sheep is controlled by environmental and genetic factors as well. But productive traits in domestic livestock are generally identified to be with multigenic inheritance. Therefore, selection for reproduction may be a long and very slow process (Nanekarani et al., 2016).

Many genes associated with reproduction are identified and can be used in breeding through marker-assisted selection. Some major genes, called fecundity genes are responsible for high prolificacy in sheep – GDF9/FecG, BMP-15/FecX, BMPR-1B/FecB (Monteagudo et al. 2009; Polley et al., 2010; Vacca et al., 2010).

The *FecB* or Booroola gene is the first major fecundity gene identified in sheep. A point mutation resulted of A → G (,A746G, p.Q249R) transition at position 746 in highly conserved region of the BMPR-1B gene, leads to increasing the ovulation rate with 1.6 and litter size by 1 to 2 extra lambs with one copy of the FecB mutation (Georgescu et al., 2011; Liu et al., 2014; Maskur et al., 2016).

BMPR-1B gene is part of the transforming growth factorβ (TGF-β) receptor superfamily. It has a coding sequence with length 1,509 bp, and it contains 10 exons. Its expression is mainly in granulose cells. BMPR-1B/FecB gene is located on chromosome 6 of the sheep genome (Mulsant et al., 2001). FecB gene is considered as a major gene which increases (Moradband et al., 2011; Karsli et al., 2012).

The aim of the present experiment was the identification of the allelic variant and estimation of genetic diversity of BMPR-1B/FecB gene in 30 animals belonging to the Northeast Bulgarian Merino sheep breed.

Materials and Methods

In the present study, were tested 30 ewes from the Northeast Bulgarian Merino sheep breed for genetic diversity in BMPR-1B/FecB gene. Approximately 3 mL blood were collected from each individual in vacuum tubes containing EDTA by the method of Miller et al. (1988). When receiving blood samples, all animal welfare requirements are met.

The experiment was carried out in the Laboratory of Genetics part of the Institute of Animal Science, Kostinbrod, Bulgaria.

The blood samples were stored at –20°C until DNA extraction. DNA was extracted from whole blood by manual commercial kit for DNA purification according to the manufacturer's instruction (QIAamp DNA Blood Mini Kit, Qiagen). After manual DNA extraction, were received 30 samples with DNA concentration of approximately 10 ng/µl. The quality of the obtained DNA was tested using gel monitoring on 1% agarose (GE Healthcare, UK) gel prepared with 1xTBE buffer (Thermo). PCR amplification was carried out in total volumes of 10 µl, containing 40 ng DNA template, 20 pM of each primer and 2× (1.5 mM MgCl2) Red Taq DNA Polymerase Master mix (VWR, Int., Belgium). The primer set was suggested by Wilson et al. (2001):

-forward primer:

5'-CCAGAGGACAATAGCAAAGCAAA-3

-reversed primer:

5'-CAAGATGTTTTCATGCCTCATCAACAGGTC-3

The polymerase chain reaction was performed using thermal cycler TC-TE (BOECO, Germany) under the following conditions: primary denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 59°C for 1 min, elongation at 72°C for 1 min. The reaction was completed by a final extension at 72C for 10 min.

The genotypes of the analyzed individuals were established using restriction fragment length polymorphism (RFLP) analysis. The digestion reaction was carried out in 10 µl final volume, containing 6 µl PCR product and 10 U/µl AvaII restriction enzyme (Jena Bioscience). PCR products were incubated at 37ºC for 16 h in thermo-block. The fragment sizes were determined using GeneRuler™ Ladder, 50 bp (Fermentas) supplied with 1 ml 6xDNA Loading dye (Thermo) on 2,5 – 3 % agarose gel, and then visualized under UV light on trans-illuminator Hi-UV^{TM} Duo Capture (HIMEDIA).

Results

PCR-RFLP is a simple, rapid, and exact method for genotyping of single nucleotide polymorphism (SNP). This technique has shown satisfactory results in the genotyping of prolific sheep (Guan et al., 2007; Polley et al., 2009).

After PCR amplification were received fragments with expected length of 190 bp in all 30 animals. Digestion with specific restriction enzyme *AvaII* produced two different alleles – the wild allele "+" and the mutant allele "B".

In tested herd were identified two of three possible genotypes – homozygous wild genotype "++" with frequency 0.93 and heterozygous genotype "B+" with frequency 0.07. The homozygous mutant genotype "BB" was not found. Results of the PCR-RFLP electrophoresis of the FecB gene after digestion with *AvaII* are presented in Figures 1 and 2. Allelic and genotypic frequencies are presented in Table 1 and Figure 3.

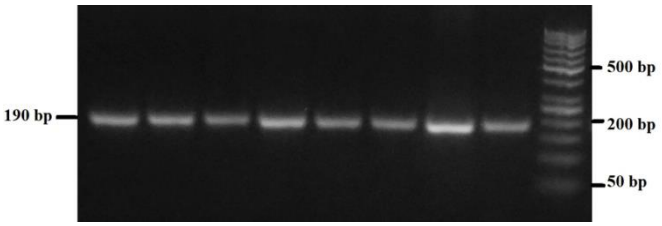

Figure 1. *Amplified product of FecB gene in Northeast Bulgarian Merino sheep breed on 2.5 % agarose gel.*

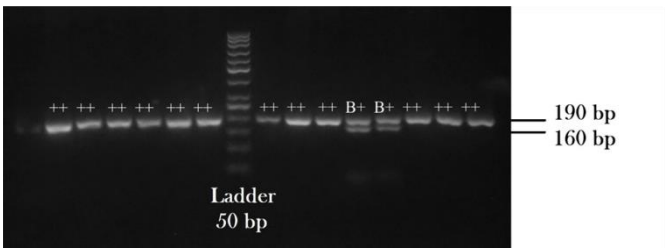

Figure 2. *Image of PCR product of the FecB mutation of the BMPR-1B gene digested with Ava II. The wild allele "+" is 190 bp, and the mutant allele "B" is 160 bp.*

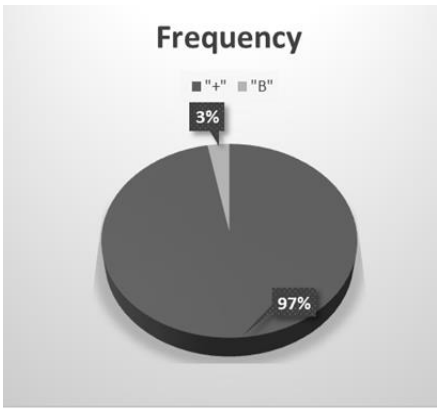

Figure 3. *Graphic of allele distribution in BMPR-1B locus.*

Table 1. *Number of tasted animals (n), number of alleles per locus (observed Ao and effecteve Ae), allele and genotype frequencies, average heterozygosity (observed Ho, expected He) and chi-square (χ2) for HWE in the examined population.*

Locus	n	Allele Number		Allele Frequencies		Genotype Frequencies			Heterozygosity		df*	χ2	P**
		A_o	A_e	"+"	"B"	"++"	"B+"	"BB"	H_o	He			
BMPR-1B	30	2.00	1.06	0.97	0.03	0.93	0.07	0.00	0.066	0.058	1	0.00	NS

* degree of freedom = 1, ** degree of probability > 0.05

The presence of mutant allele "B" could be due to the use of sires from Australian Merino, Booroola, and Il de France to improving the Bulgarian Merino sheep breed hence hyper-prolific phenotype was first discovered in the Australian Merino breed (Stancheva et al., 2017).

Discussion

Apart from the Australian merino, the mutation has been found in Indian sheep breeds - the most fertile Garole, and Kendrapada sheep, India's second most fertile sheep breed (Dash et al., 2017). In other study were tested two Indian sheep breeds. In Nellore sheep breed were found the three genotypes with a frequency of mutant genotype "BB" – 0.03, heterozygous "B+" - 0.12% and wild "++" 0.85. In ewes Deccani results similar to this study were reported, two genotypes were found, but with a higher frequency of the heterozygous genotype "B+" - 0.13% (Praveena et al., 2017).

Iovenko et al. (2020) have studied the polymorphism of FecB in Askanian Fine-Fleeced, Askanian Meat-and-Wool, Askanian Karakul animals, and hybrids of Askanian Fine-Fleeced × Texel and found the absence of polymorphism. BMPR-1B gene in Iranian Arabic sheep breed (Mohammadi, 2016) and in Sangsari sheep breed (Jamshidi et al., 2013) was also monomorphic.

In Chinese Merino prolific meat strain were reported the presence of the three different Booroola genotypes ("BB", "B+" and "++"). The authors reported a positive relationship between mutation of FecB gene and litter size of ewes with "BB" genotype (2.84±0.74) which was significantly higher than ewes with "++" genotype (1.23±0.68) ($P < 0.01$,). The ewes with the "BB" genotype had 0.5 lambs more than ewes with the "B+" genotype, but the difference was not statistically significant. The lambs with genotypes "BB" and "B+" had higher body weight than those with genotype "++" at 90th day after birth (18.6±3.70 kg, 18.0±3.71 kg versus 15.6± 2.22 kg, $P < 0.05$) (Guan et al., 2007).

The established polymorphism in the BMPR-1B/FecB gene shows that the research of this locus in the Bulgarian sheep breeds needs to be studied in detail in order to be included in the breeding programs.

CONCLUSION

Identification of genes associated with quantitative trait loci would help to improve the quality and diversity of production in the livestock industry, through optimizing the breeding programs. The results in this study showed the presence of a mutation in the BMPR-1B/FecB gene in the investigated herd of 30 ewes from the Northeast Bulgarian Merino sheep breed. After PCR-RFLP analysis it was found the wild allele "+" and the mutant allele "B" with frequencies 0.97 and 0.03, respectively. According to the results the genotype frequencies were as followed: wild homozygous genotype "++" 0.93 and heterozygous genotype "B+" 0.07, respectively. The observed (Ho) and expected heterozygosity (He) were 0.066 and 0.058, respectively. It can be concluded that the studied herd is in Hardy-Weinberg equilibrium. PCR-RFLP technique can be used to detect and genotype the *FecB* gene clearly. This is the first study in Bulgarian sheep breeds where the presence of Booroola mutation was found.

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