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DETECTION OF POLYMORPHISM IN FecB GENE IN BULGARIAN SHEEP BREEDS

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ABSTRACT

The purpose of the present work was to identify allelic variants of ovine FecB gene in three fine fleece sheep breeds in Bulgaria – Askanian, Caucasian and Karnobat Merino. Blood samples were collected from 30 animals of each breed (total of 90 samples). Genomic DNA was extracted using commercial kit for DNA purification from whole blood. After PCR amplification of FecB gene with specific primer set a PCR fragment with length 190 bp was obtained. Genotypes were determined by restriction fragment length polymorphism (RFLP) method with AvaII restriction enzyme. The locus of Boroola gene was found to be monomorphic. Only allele "+" was found and only genotype "++", respectively. According to the results the investigated animals were non-carriers of the mutation.

Key words: sheep, PCR, RFLP, FceB, polymorphism.

Introduction

In recent years improving of reproduction traits in livestock has become of great interest, especially in sheep breeding where the increasing of litter size could lead to large profit for the producers. Prolificacy and litter size are an important economic traits in sheep breeding, and they are controlled by both genetic and environmental factors. Most sheep breeds have one or two lambs at each lambing (Bindon and Piper, 1986, Fugarti, 2009).

The increasing the prolificacy by using traditional breeding strategies could be very slow process (N. Govardhana Sagar et al., 2017). That is way the identification of candidate genes associated with reproduction can be utilized in breeding trough marker assisted selection (MAS). Reproductive traits are often suggested as main target for MAS for their low heritability and the fact that the trait can be measured only in one sex (Ghaffari et al., 2009).

Many studies have indicated that the ovulation rate and litter size can be genetically regulated by a group of different genes, collectively named as fecundity (Fec) genes (Al-Barzinji and Othman, 2013, Davis et al., 1982). In different sheep breeds, four major genes affecting fecundity have been identified: BMPR1B/FecB (bone morphogenetic protein receptor type 1B), BMP15/FecX (bone morphogenetic protein 15), GDF9 / FecG (growth differentiating factor 9) and B4GALNT2/FecL (Beta-1,4-N-Acetyl-Galactosaminyltransferase 2) (Galloway et al., 2000; Mulsant et al., 2001; Hanrahan et al., 2004; Drouilhet et al., 2013).

FecB or the Booroola gene is a single autosomal gene which influences on litter size and ovulation rate. It is co-dominant for ovulation rate and partially dominant for litter size (Piper & Bindon 1982; Davis et al. 1982). The mutation induces precocious maturation of ovarian follicles by increasing the sensitivity of the follicles to follicle stimulating hormone (FSH) without increasing circulating FSH concentrations. This phenotype has a Mendelian pattern of segregation indicating that it is caused by a major gene, which has additive effects on ovulation rate and is dominant for litter size (Ghaffari et al., 2009; Kumar et al., 2013).

BMPR1B locus is situated on chromosome 6 of sheep genome (Ovis Aries). The effect of FecB mutation is additive for ovulation rate and each copy increases ovulation rate by about 1.6 (Piper and Bindon, 1996).

The Booroola mutation (FecB) is thought to be originated from the Garole breed in India which carries the FecB mutation (Davis et al., 2002).

The present study was conducted in order to be identified the allelic variants of FecB gene in three Bulgarian fine fleece sheep breeds – Askanian, Caucasian and Karnobat Merino.

Materials and methods

Approximately 3 mL blood were collected from three fine fleece sheep breeds – Askanian, Caucasian and Karnobat Merino, each represented of 30 individuals. Blood samples were collected from v. jugularis in vacuum tubes containing EDTA as anticoagulant. The investigation was carried out in the Laboratory of Genetics, the University of Forestry.

The blood samples were storage 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 (Illustra Blood GenomicPrep DNA Purification Kit, GE Healthcare). DNA concentration of each sample was verified by spectrophotometer Biodrop. The quality of the obtained about 10–50 ng DNA was tested using gel monitoring on 1% agarose (Bioline) gel prepared with TBE buffer (Jena Bioscience).

PCR amplifications were carried out in total volumes of 10 µl for each sample, containing 4 µl DNA template, 0.2 µl dd H2O, 0.4 µl primer and 5 µl of 2 × (1.5 mM MgCl2) MyTaqTM HS Red Mix 2x (Bioline). The primer set was suggested by Wilson et al. (2001):

- forward primer: 5'-CCAGAGGACAATAGCAAAGCAAA-3
- reversed primer: 5'-CAAGATGTTTTCATGCCTCATCAACAGGTC-3

PCR reactions were accomplished by thermocycler QB-96 (Quanta Biotech) 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 final extension at 72°C for 10 min and storage at 10°C forever.

The genotypes of all investigated animals were established using RFLP (restriction fragment length polymorphism) analysis. All PCR products were digested in 10 µl final volume of each sample, containing 6 µl PCR product, 2.5 µl ddH_2O, 0.5 µl restriction enzyme AvaII (jena Scientific) and 1 µl enzyme buffer. The PCR products were incubated in thermostat at 37°C for 12h. The fragment sizes were determined using Ready to Use DNA Ladder, 50 bp (Thermo) on 2,5% agarose (Bioline) gel and stained by RedGel Nucleic Acid Stain (Bioline). The obtained PCR products and restriction fragments were visualized under UV light.

Results and Discussion

In this study a total of 90 individuals from three fine fleece sheep breeds were studied for the very first time on FecB gene in Bulgaria. After DNA extraction it was received samples with concentration approximately 10-15 ng (Figure 1). The PCR amplification revealed fragments with expected length of 190 bp in all 90 investigated samples.

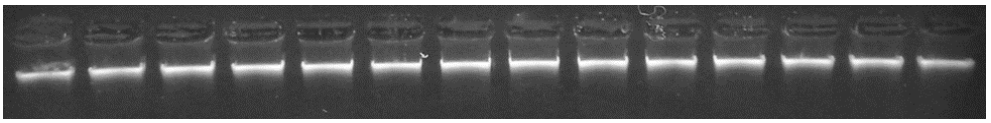

Figure 1: Gel Monitoring for Testing of DNA Samples

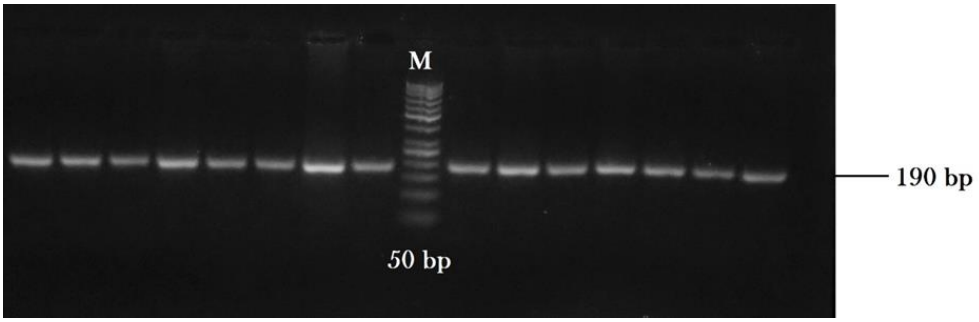

Figure 2: Restriction analysis of PCR products of FecB gene with AvaII restriction enzyme on 2.5% agarose gel

After digestion with AvaII authors reported that homozygous carriers for FecB gene should produce a 160 bp band (BB), the non-carrier should produce a 190 bp band (++), whereas heterozygotes should produce both 160 and 190 bp bands (B+) (Gootwine et al. 2008; Hua and Yang 2009).

In present work all investigated animals had the wild genotype "++" which means that only the presence of allele "+" was found. In Table 1 are presented the allele frequencies, genotype frequencies and heterozygosity which were similar in this case and with no variations.

Table 1: Breed, allele number, allele frequency, genotype frequency and heterozygosity

Breed	Allele number		Allele frequency		Genotype frequency			Heterozygosity	
	n_a	n_e	'+'	'B'	"++"	"B+"	"BB"	H_o	H_e
Askanian	1.00	1.00	1.00	0.00	1.00	0.00	0.00	0.00	0.00
Kawkasian merino	1.00	1.00	1.00	0.00	1.00	0.00	0.00	0.00	0.00
Karnobat merino	1.00	1.00	1.00	0.00	1.00	0.00	0.00	0.00	0.00

The result in this study are in agreement with Ghaffar et al., (2009). They studied Shal Sheep and reported for presence of only one allele "+" and one genotype "++", respectively.

Al-Thabhawee et al., (2014) investigated local sheep breed in different areas in Iran and announced that after Ava II digestion revealed only one band of 190 bp product (wild type form) in all tested animals, and none of them were carrying gene mutation form.

Al-Barzinji and Othman, (2013) studied another region of FecB gene by PCR-RFLP technique in five Iraqi sheep breeds. Although they did not find polymorphism in investigated part of the gene.

On the contrary of our work Feng Guan et al (2007) studied nine sheep breeds and strains for polymorphism in FecB gene by PCR-RFLP method. Their results showed that the polymorphism frequencies of FecB gene were significantly imbalanced in these breeds or strains. The Hu sheep were all homozygous carriers (BB). In the Chinese Merino prolific meat strain, the genotype frequencies of B, B+ and ++ were 51%, 30% and 19%, respectively, whereas all the other flocks had only the wild-type (++) genotype.

Joyabrata et al (2011) investigated the FecB locus in the Indian Bonpala Sheep and found the three possible genotypes - wild type (++, 0.02), heterozygous (B+, 0.23), and mutant (BB, 0.75).

Polymorphism in FecB gene was announced by Asadpour et al., (2012) in Zei sheep breed with presence of two different genotypes B+ and ++ with frequencies 1,47 % and 98,53 %, respectively.

Praveena et al., (2017) also studied FecB gene in two sheep breeds in India. Among 40 Deccani sheep breed blood samples, they detected two genotypes - heterozygous genotype "B+" and homozygous "+/+". Nellore sheep breed they found all three possible genotype "BB", "B+" and "++" with frequencies 2.5 %, 12.5 % and 85 %, respectively.

Conclusion

Results indicate that PCR-RFLP method is helpful in genotyping of investigated gene. It may be concluded that the FecB gene is monomorphic in all 90 investigated animals from the three fine fleece sheep breeds eared in Bulgaria – Askanian, Caucasian and Karnobat Merino. Only the allele "+" and the genotype "++" with a frequency of 1.00 were detected. All animals were defined as non-carrier of Boroola mutation.

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