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PCR-RFLP ANALYSIS OF CALLIPYGE GENE (CLPG) IN KARAKACHAN SHEEP BREED

I. DIMITROVA* and M. BOZHILOVA-SAKOVA
University of Forestry, Agronomy Faculty, BG-1756 Sofia, Bulgaria

Abstract

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One of the genes studied in relation to improve the meat productivity of sheep is callipyge (CLPG). This research aimed to identify the genetic polymorphism in callipyge gene in sheep from Karakachan breed. Blood samples were taken from *v. jugularis* of twenty-two ewes and three rams from private herd in town Sapareva Banya. Genomic DNA was extracted from whole blood. By using of PCR reaction were amplified 426 bp fragments from CLPG gene. PCR-RFLP analysis of this part of callipyge gene was carried out by restriction enzyme *BsmF1*. It was detected only homozygous genotype AA, respectively CLPG locus was found to be monomorphic in this population.

Key words: sheep, Karakachan breed, callipyge gene (CLPG), restriction enzyme *BsmF1,* PCR-RFLP method

Introduction

Natural mutations exist in sheep that affect muscle growth and development, and the exploitation of these mutations in breeding strategies has the potential to significantly improve lamb-meat quality. The best-documented mutation for muscle development in sheep is callipyge (CLPG), which causes a postnatal muscle hypertrophy that is localized to the pelvic limbs and loin with little or no effect on anterior skeletal muscles. Callypige phenotype in sheep is also called "beautiful buttocks". The hypertrophy develops only paternal heterozygous animals (mutant type allele inherited from father and wild type allele in inherited from mother). The maternal heterozygous and homozygous animals have normal phenotype, this novel mode of non-mendelian pattern of inheritance are named "polar overdominance" (Cockett et al., 1996; Vuocolo et al, 2007; Rejduch, 2008; Tellam et al., 2012). Computerized tomography analysis of live lambs showed that carrying the callipyge mutation increased *m. Longissimus dorsi* and *m. Quadriceps femoris* by about 30% along with increased tissue density (Gootwine et al., 2004). The locus of CLPG gene was mapped to the telomeric region of ovine chromosome18, within a cluster of imprinted genes (Cockett et al., 1994; Tellam et al., 2012).

In Bulgaria there are more than thirty-four sheep breeds (Nikolov et al., 2011), but information about their genetic diversity, particularly for genes associated with meat traits is very limited (Georgieva et al., 2015; Hristova et al., 2015; Bozhilova-Sakova and Dimitrova, 2016; Dimitrova et al., 2016).

Karakachan breed is one of the oldest sheep breeds in Europe, pure form of coarse wool type Tzakel in Bulgaria. Animals are small, vigorous, strong-boned with short legs and resistant to unfavorable climatic and feeding conditions, and diseases (Dimitrov and Dimitrova, 1995).

This research aimed to identify the genetic polymorphism in callipyge gene in sheep from Karakachan breed.

Materials and Methods

Animals and blood collection

In present study were involved animals of a private herd in town Sapareva Banya, Bulgaria, from indigenous Bulgarian sheep breed Karakachan – twenty-two ewes and

**Corresponding author:* ivonna_dimitrova@yahoo.co.uk

three rams. Blood samples were collected from *v. jugularis* in 3 ml vacuum tubes containing EDTA as anticoagulant and transported to the laboratory. The investigation was carried out in the Laboratory of Genetics, the University of Forestry.

DNA extraction

The blood samples were storage at –20°C until DNA extraction. Genomic 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). The DNA concentration of each sample was determined by spectrophotometer Biodrop. The quality of the obtained about 10–50 ng DNA was tested using gel monitoring on 1% agarose (Healthcare) gel prepared with TBE buffer (Thermo).

PCR-RFLP analysis

PCR amplifications were carried out in total volumes of 10 µl, containing 4 µl DNA template, 0.2 µl dd H2O, 0.4 µl of each primer (Bioneer) and 5 µl of 2×(1.5 mM MgCl2) Red Taq DNA Polymerase Master mix (VWR, Int., Belgium). For CLPG locus was used primer set suggested by Freking et al. (2002): forward primer: 5′-TGA AAA CGT GAA CCC AGA AGC-3′ and reverse primer: 5′-GTC CTA ATA AGG TCC TCT CG-3′. PCR reactions were accomplished by thermocycler QB-96 (Quanta Biotech) under the following conditions (Nanekarani et al., 2014): primary denaturation at 95°C for 4 min, followed by 35 cycles of denaturation at 94°C for 20 s, annealing at 58°C for 30 s, 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 separately in 10 µl final volume, containing 6 µl PCR product, 2.5 µl ddH2O, 0.5 µl restriction enzyme *BsmF1* (New England BioLabs) and 1 µl enzyme buffer. The digestion reactions were carried out at 65°C for 1 h in thermo block. The fragment sizes was determined using GeneRuler™ Ladder, 50 bp (Thermo) supplied with 1 ml 6xDNA Loading dye on 2% agarose (Healthcare) gel and stained by 10000x RedGel TM Nucleic Acid Stain (Biotium). The obtained PCR products and restriction fragments were visualized under UV light.

Results and Discussion

After PCR reaction of all twenty-five Karakachan animals were amplified 426 bp fragments from CLPG gene (Figure 1).

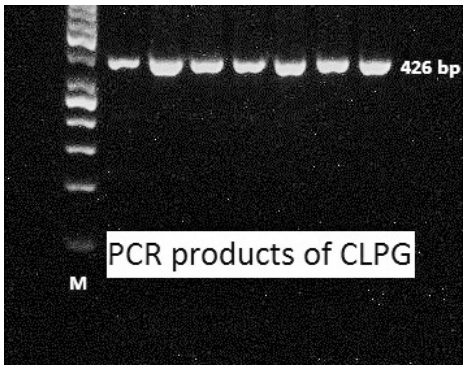

Fig. 1. Amplified 426 bp fragments of CLPG gene in sheep

The *BsmF1* restriction enzyme digests the PCR products and two alleles were detected – wild allele A (with three fragments of 278 bp, 117 bp and 31 bp) and mutant allele G (with two fragments 395 bp, 31 bp). As a result three possible genotypes could be formed – genotype AA (three fragments – 278 bp, 117 bp and 31 bp), genotype AG (four fragments – 395 bp, 278 bp, 117 bp, 31 bp) and genotype GG (two fragments – 395 bp, 31 bp), respectively (Gabor et al., 2009).

In present study was detected only homozygous genotype AA (Figure 2). Genotypes AG and genotype GG were not determined in animals from the studied herd of Karakachan sheep breed. CLPG locus was found to be monomorphic in this population.

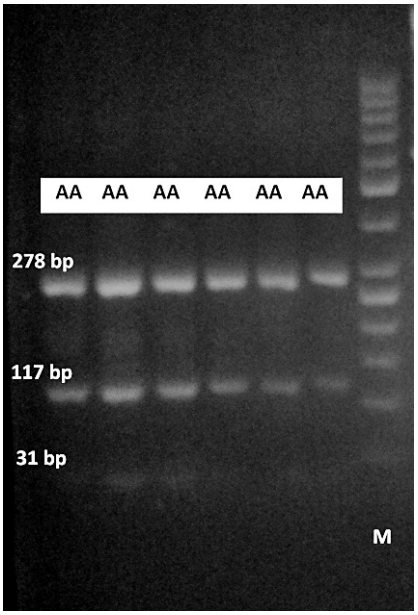

Fig. 2. Restriction fragments of CLPG gene in sheep from Karakachan breed digested with enzyme *BsmF*1, observed on 2 % agarose gel

Results in this study are in agreement to other investigations on CLPG sheep gene. Gabor et al. (2009) reported similar results for animals from breeds Lacaune, Tsigai, Improved valachian, East Friesian and crossbreed Tsigai and Lacaune. Alakilli (2015) analyzed the callipyge gene in two Saudi sheep breeds – Najdi and Harri, which were monomorphic for genotype AA of CLPG locus. Nanekarani et al. (2014) also observed in Iranian Lori sheep only AA genotype, which suggests that the Lori sheep also were monomorphic for callipyge locus. Quanbari et al. (2007) reported absence of diversity in CLPG in experimental flock of Afshari breed. Kumar (2012) reported that all studied samples from animals of Indian breeds Avikalin, Bharat Merino, Nellore, Chokla and Mapura were monomorphic for CLPG gene and only wild allele A was detected.

This study disagrees with Jackson et al. (1997), which reported presence of the mutant allele G in sheep of breeds Dorset, Rambouillet and Hampshire.

Conclusion

In this study was detected only homozygous genotype AA and allele A in the investigated fragment of CLPG locus, which was found to be monomorphic in this population of Karakachan sheep breed.

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