Genetic polymorphism study of growth hormone gene (GH1) from exons 2 and 3 in autochthonous Carpatina goat breed

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Abstract. The goats have specific characteristics, as a result of their great adaptability to the environment and food. Considering the association between GH gene polymorphism and the growth traits, the present study aimed to highlight the presence of polymorphisms on exons 2 and 3 of the GH1 gene in Crapatina breed and also the genotype and allele frequencies. Using PCR-RFLP technique were found 3 genotypes in this population: AA (uncut 422 bp fragment), BB (366 bp and 56 bp fragments) and AB (422, 366, and 56 bp fragments). The AA genotype frequency was 7%, 48% for the genotype AB and 45% for genotype BB. Also, in this population gene A appeared with a frequency of 31% and gene B with a frequency of 69%.

Key Words: gene polymorphism, gene frequency, genotype frequency, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), HAE III enzyme.

Introduction. Due to the large capacity of flexibility in adapting to the environment, the goats show special features. Goat rearing it is a very common practice in the entire world, due to the low costs for food and care, but also for their milk and meat production (Paim et al 2019). The growth hormone (GH) is associated with growth traits and also milk production traits. The gene play an important role in some biological processes as reproduction, animal breeding, and more complexe processes like lactation and metabolism (Alakilli et al 2012; Amie Marini et al 2012). Several studies on ruminants reveal the gene effect on the growth of some tissues, including bone tissue, muscular tissue and adipose tissue (Arango et al 2014).

Growth hormone is a peptide encoded by a single gene with 2.5 kb lenghts comprises of five exons and four introns. In goats, the gene was mapped on the short arm of chromosome 19 (*Capra hircus* 19q22). The growth hormone is produced by the somatotroph cells of the anterior lobe of the pituitary gland (Mahrous et al 2018; Othman et al 2015). Due to the influence on growth and body mass of the animals, some studies reveal that beside the GH gene, there are insulin–like growth factor-1, IGF-1 (Amie Marini et al 2012; Othman et al 2015). IGF-1 plays an key-role in the processes of growth, lactation, metabolism by stimulating anabolic processes like cell proliferation, skeleton development, hair growth and even protein synthesis (Alakilli et al 2012; Luo et al 2019; Sankhyan et al 2019).

Considering the essential role of GH gene in animal growth and development, this gene can be used like candidate gene for polymorphism study and its associations with growth traits. The sequence of goat pituiatary GH cDNA was reported by Yamano et al (1988) and Yato et al (1988) and the GH gene sequence was described by Kioka et al (1989). The coding sequence predicted from the gene sequence correspond to the goat GH cDNA sequence. In goats, GH gene and polymorphism expression at gene and protein level are allready known, but there is a very poor amount of data regarding the changes and position in GH sequence. The goat GH gene of 2544 bp consist in four intervening introns and five exons (Amie Marini et al 2012).

The five exons of goat GH gene have been successfully studied using PCR-RFLP technique, using 5 fragments of goat GH gene: GH1, GH2, GH4, GH5 și GH6 (Yao et al 1996; Silveira et al 2008).

The genetic polymorphism of GH gene can be identified by multiple techniques. One of the most used technique is the PCR-RFLP technique. This thechnique was successfully used for identifying nucleotide sequence variation in amplified DNA and also can detect single base substitutions in enzymatic restriction sites (Amie Marini et al 2012; Kusza et al 2016; Meydan et al 2017).

The objectives of the present study were to identify the growth hormone gene exons 2 and 3 polymorphism (GH1 locus) in Carpatina goats and to establish the gene and genotype frequency in the studied population. For the future, we intend to analyze others loci on GH gene: GH2 and GH6.

Material and Method. For this study we used 29 Carpatina goats (23 females and 3 males). Blood samples were taken from the jugular vein and the samples were colected in tubes containing K_2DTA as an anticoagulant. Genomic DNA was extracted using extraction kit (Quick DNA Miniprep Plus Kit, 50 preps, Biozyme). The extracted DNA had mean purity of 1.90 and the concetration was 50 ng/µL. A PCR amplification reaction was performed using specific primers.

F: CTC TGC CTG CCC TGG ACT
R: GGA GAA GCA GAA GGC AAC

The PCR cocktail consisted of 5x FIREPol Master Mix (Solis BioDyne) 5 µL, 1 µL of each primer, ultrapure H_20 and 2 µL of goat DNA.

The reaction was run at 95^0C for 5 minutes, 35 cycles of 95^0C for 1 minute, 58^0C for 45 seconds and 72^0C for 1 minute and final extension at 72^0C for 8 minutes. The protocol proposed by Mahrous et al (2018) was used and it was slightly modified (primers aligning temperature was modified from 54^0C to 58^0C). The next step was to analyse the 422 bp GH1 polymorphism.

The PCR products were digested using restriction enzyme HAE III in order to highlight the polymorphism. For this step we used 15 µL of PCR product, 1 µL of HAE III enzyme, 2.5 µL of Buffer, 6.5 µL: of H_2O, mentionig that the PCR product was digested with HAE III restriction enzyme for 5 hours at 37^0 C.

The digested product was separated by electrophoresis in 2.5% agarose gel using a slow voltage (70 V).

Results and Discussion. The electrophoretic pattern higlight the AA homozygous genotype with undigested one fragment at 422 bp in 2 tested animals, BB homozygous genotype with 2 digested fragments at 366 bp and 56 bp in 14 tested animals and the AB heterozygous genotype with three digested fragments at 422 bp, 366 pb and 56 bp in 13 tested animals from the studied goat population (Figures 1-3).

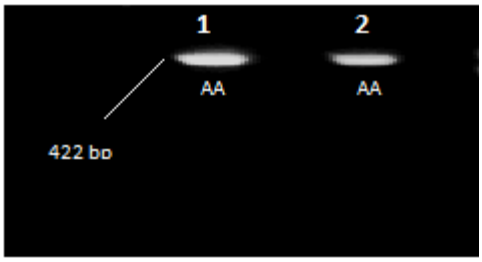

Figure 1. Electrophoretic pattern of PCR amplified fragment (undigested fragment at 422 bp) of GH gene with HAE III enzime. Lane 1 and 2: AA homozygous genotype in 2 tested animals.

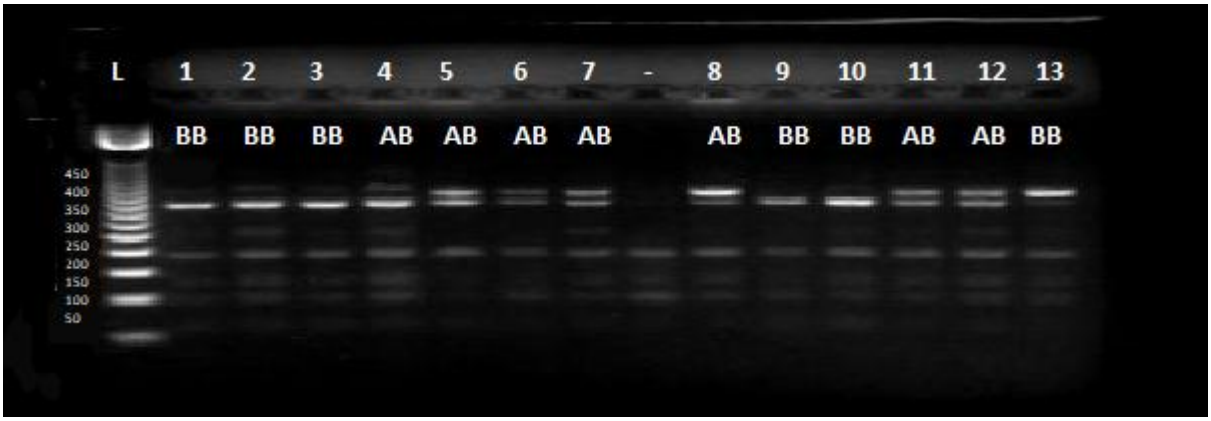

Figure 2. Electrophoretic pattern of PCR amplified fragment of GH gene with HAE III enzime. Lane 4, 5, 6, 7, 8, 11, 12: AB heterozygous genotype. Lane 1, 2, 3, 9, 10, 13: BB homozygous genotype.

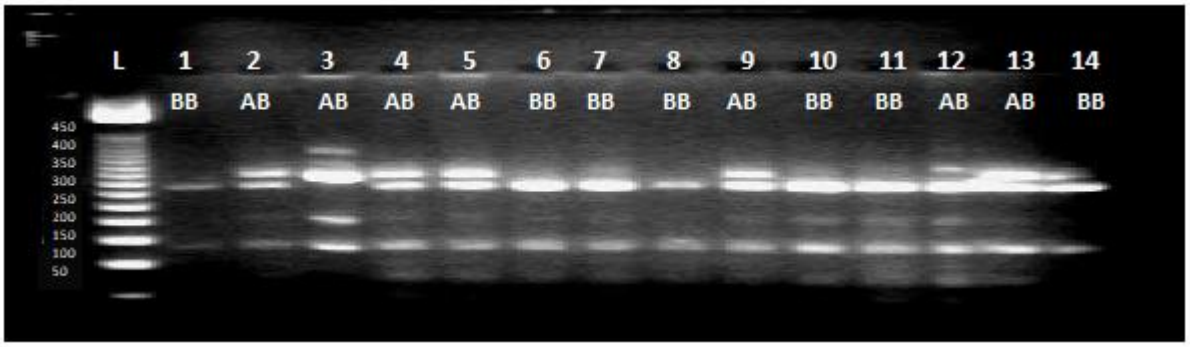

Figure 3. Electrophoretic pattern of PCR amplified fragment of GH gene with HAE III enzime. Lane 2, 3, 4, 5, 9, 12, 13: AB heterozygous genotype. Lane 1, 6, 7, 8, 10, 11, 14: BB homozygous genotype

After studying the genotypes at GH1 locus for all the studied animals, genotype and allele frequency was calculated. As shown in Table 2 the AA genotype had the lowest frequency (0.07); AB genotype frequency was 0.48 and BB genotype frequency was 0.45 (Table 1).

Table 1

Genotype frequency of the GH1 gene in Carpatina goats

Genotype	*Frequency*
AA	0.07
AB	0.48
BB	0.45

The A allele frequency (0.31) was lower then the B allele frequency (0.69) in the studied goat population (Table 2).

Table 2

Allele frequency of the GH1 gene in Carpatina goats

Allele	*Frequency*
A	0.31
B	0.69

Also, the literature cites some cases were the B allele show a higher frequency in the population compared to the A allele (Mahrous et al 2018). As we have shown above, in our Carpatina goat population the AA genotype was detected, compared to the literature where no AA genotype was detected, as follows: in Barki breed the A allele frequency was 0.47 and B allele frequency was 0.53, genotypes frequencies: AA- 0, AB -0.95, BB – 0.05; in Damascus breed the A allele frequency was 0.45 and B allele frequency was 0.55, genotypes frequencies: AA - 0, AB – 0.90, BB – 0.10; in Zaraibi breed the A allele frequency was 0.47 and B allele frequency was 0.53, genotypes frequencies: AA - 0, AB – 0.95, BB – 0.05.

Conclusions. In the present study, PCR-RFLP technique proved to be a convenient technique for screening gene polymorphism. In our Carpatina goat breed population all three genotypes were detected (AA, AB, BB). The highest frequency was for AB genotype (0.48) followed by BB genotype (0.45) and the lowest frequency was for AA genotype (0.07). Regarding the gene frequency we can conclude that the B allele frequency was higher in our goat population.

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