Identification of 332G>A Polymorphism in Exon 3 of the Leptin Gene and Partially Effects on Body Size and Tail Dimension in Sanjabi Sheep

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Abstract : The objective of the present study was to determine the polymorphism in the leptin (332G>A) and its association with biometric traits in Sanjabi sheep. For this purpose, blood samples from 96 rams were taken, and tail length, width tail, circumference tail, body length, body width, and height were simultaneously recorded. PCR was performed using specific primer to amplify 463 bp fragment including exon 3 of leptin gene, and PCR products were digested by Cail restriction enzymes. The 332G>A (at 332th nucleotide of exon 3 leptin gene) that caused an amino acid change from Arg to Gln was detected by Cail (CAGNNNCTG) endonuclease, as the endonuclease cannot cut this region if G nucleotide is located in this position. Three genotypes including GG (463), GA (463, 360and 103 bp) and GG (360 bp and 103 bp) were identified after digestion by enzyme. The estimated frequencies of three genotypes including GG, GA, and AA for 332G>A locus were 0.68, 0.29 and 0.03 and those were 0.18 and 0.82 for A and G alleles, respectively. In the current study, chi-square test indicated that 332G>A positions did not deviate from the Hardy–Weinberg (HW) equilibrium. The most important reason to show HW equation was that samples used in this study belong to three large local herds with a traditional breeding system having random mating and without selection. Shannon index amount was calculated which represent an average genetic variation in Sanjabi rams. Also, heterozygosity estimated by Nei index indicated that genetic diversity of mutation in the leptin gene is moderate. Leptin gene polymorphism in the 332G>A had significant effect on body length (P<0.05) trait, and individuals with GA genotype had significantly the higher body length compared to other individuals. Although animals with GA genotype had higher body width, this difference was not statistically significant (P>0.05). This non-synonymous SNP resulted in different amino acid changes at codon positions111(R/Q). As leptin activity is localized, at least in part, in domains between amino acid residues 106-1406, it is speculated that the detected SNP at position 332 may affect the activity of leptin and may lead to different biological functions. Based to our results, due to significant effect of leptin gene polymorphism on body size traits, this gene may be used a candidate gene for improving these traits.

Keywords : body size, Leptin gene, PCR-RFLP, Sanjabi sheep
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