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Research Article

THE EVALUATION OF SECOND INTRONS AND THIRD EXON POLYMORPHISM OF LEPTIN AND ITS RELATIONSHIP WITH SOME GROWTH PARAMETERS BY GENE SEQUENCING IN LORI BAKHTIARI RAM LAMBS

Mansour Shokri and *Mostafa Faghani
Department of Animal Science, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran
**Author of Correspondence*

ABSTRACT
This study was performed for evaluation of second introns and third exon polymorphism of leptin and its effect on growth parameters by gene sequencing in Lori Bakhtiari ram lambs. For this reason a total of 86 Lori Bakhtiari sheep breeds were used. All lambs were identified at birth, and birth weights, as well as sex, birth type, and pedigree information was recorded. Polymerase chain reaction (PCR) products were subjected to single strand conformation polymorphism (SSCP) denaturation and polyacrylamide gel electrophoresis. Result showed that there were no significant differences for S3201G/A and S3208G/T according to weight traits at the birth stage and 1-3,3-6,6-9 and 9-12 month. The relationship between weight traits with S3405A/T marker mutation showed that significant effects on weight at the birth stage (4.08±015 g.d) and body weight gain until one to three months (295.94±10.19 g.d) respectively. In conclusion we could confirm that potential usefulness of GH and LEP genes in marker assisted selection programs of sheep breeding.

Keywords: *Leptin, Growth Parameters, Introns and Exon, Gene, Lori Bakhtiari Sheep*

INTRODUCTION
Genetic investigation to achieve the existing biodiversity and differences among the important sheep breeds is an essential fact to facilitate the conservation program in an effective and meaningful way. Genes affecting polygenic traits and characterizing meat or wool production are difficult to identify (Clement *et al.*, 1998). However, a number of potential candidate genes have been recognized which are selected on the basis of a known relationship between physiological or biochemical processes and these traits could be tested as quantitative traits loci (Machugh *et al.*, 1998). Among these one of the candidate genes for marker assisted selection is leptin (Nassiry *et al.*, 2008). Leptin is one of hormones that have been recognized to have a major influence on energy balance (Tahmoorespur and Ahmadi, 2012). Leptin is a mediator of long term regulation of energy balance, suppressing food intake and thereby inducing weight loss. It is a hormone produced by adipocytes that may signal the brain as a satiety factor (Spicer and Francisco, 1998). Leptin is an adipokine peptide hormone produced by fat cells, particularly in white adipose tissue. A number of studies in rodents have shown that leptin can affect endocrine function, especially the secretion of hormones from the anterior pituitary, ovary and adrenal gland (Bornstein *et al.*, 1997). Serum leptin sensitivity to energy balance is reduced during periods of negative energy balance in sheep. The transition from the pre ruminant to ruminant state of lambs contributes too many changes in circulating concentration of insulin, thyroid hormones and leptin (Tokuda *et al.*, 2001). Very little information is currently available to compare different sheep population. Leptin gene polymorphism exon 3 was studied and found that with growth traits are significantly affected by the genotypes in Kermani sheep by Shojaei *et al.*, (2010) and suggested that polymorphism in Leptin gene loci may be used as a selective marker to improve growth traits in future (Qureshi *et al.*, 2015). The exon 3 of leptin a gene also studied in Iranian Makoei sheep and Five SSCP patterns were identified by Hashemi *et al.*, (2011). The aim of this paper was to investigate and evaluate second introns and third exon polymorphism of leptin gene and its relationship with some growth parameters by gene sequencing method in Lori Bakhtiari ram lambs.

MATERIALS AND METHODS

A total of 86 samples from Lori Bakhtiari sheep breeds of the Chahrmahal and Bakhtiari province of Iran were selected for this study. All lambs are identified at birth, and birth weights, as well as sex, birth type, and pedigree information are recorded. During the suckling period, lambs are fed with their mothers' milk and are also allowed dry alfalfa after 3 weeks of age.

Animal Genomic Screening

Blood samples (approximately 3–4 ml) were obtained from 86 Lori Bakhtiari sheep breeds from Shooli agriculture research center of Shahrekord, Iran and stored in ethylene diamine tetra acetic acid (EDTA) coated tubes. Genomic DNA was extracted from 0.3 ml blood using the genomic DNA purification kit (Fermentas, CinnaGen Inc., Tehran, Iran) according to manufacturer's instructions. The quality and quantity of extracted DNA was measured by 0.8% agarose gel electrophoresis. DNA amplification of the LEP gene was achieved by polymerase chain reaction (PCR). Two PCR primers, LEP‐up (5‐AGGAAGCACCTCTACGCTC‐3) and LEP‐dn (5′‐CTTCAAGGCTTCAGCACC‐3′), targeting a fragment of 471 bp were employed as described (Zhou *et al.,* 2009). The PCRs were carried out in 50 µl volumes using PCR Master Mix kit (Cinna Gen Inc., Tehran, Iran) containing 2.5 U of Taq DNA polymerase in reaction buffer, 4 mM MgCl2, 50 µM of each of dATP, dCTP, dGTP, and dTTP, 0.5 µM of each primer, and about 100 ng of extracted DNA as a template. The thermal profile consisted of 2 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 59°C, and 30 s at 72°C, with a final extension of 5 min at 72°C. Amplification was carried out in a Master cycler (Eppendorf, Homburg, Germany).

Statistical Analysis

For the association studies, the traits of interest were analyzed using the general linear model (GLM) procedure of the SAS program, according to the following statistical model:

Yijklm = µ +sexi+mounthj+biparousk+lpl+eijklm

Whereas: yijklm =Treatments, µ= Average, sexi= Animal sex, mounthj=Month of the birth, biparousk= Single or multiple birth, lp_l= The effect of leptin gene mutation and eijklm= Effect of error.

RESULTS AND DISCUSSION

Result

Evaluation of the relationships between leptin markers with weight traits was done with 86 samples. Levels of significance, least squares means, and standard errors are reported in Tables 1 and 2.

DNA Extraction

The DNA extraction and separation was performed using (Fermentas, CinnaGen Inc., Tehran, Iran) purification kits at Islamic Azad university Shahrekord branch Biotechnology lab (figure 1 and 2).

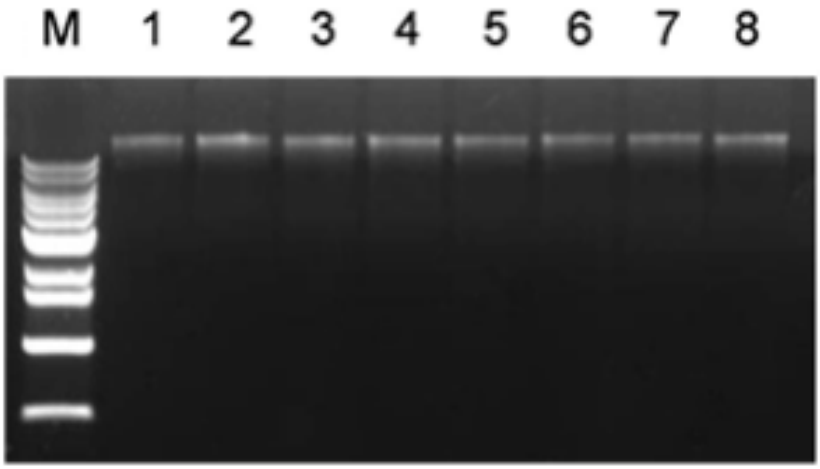

Figure 1: Gel Electrophoresis of Samples DNA Extracted Image

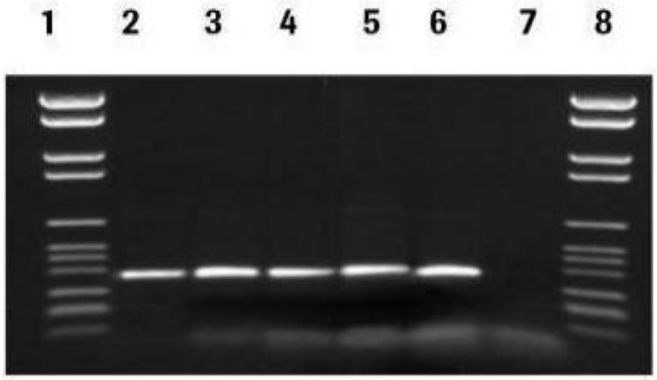

Figure 2: Leptin Gene Amplification Using Polymerase Chain Reaction by Specific Primers

The investigation of relationship between leptin markers with weight traits showed that there were no significant differences for S3201G/A and S3208G/T according to weight traits at the birth stage and 1-3,3-6,6-9 and 9-12 month. The average difference markers on growth traits such as (weight at the birth and body weight gain until one to three month was seen for S3405A/T, S3515T/G and S3596C/G markers. The relationship between weight traits with S3405A/T marker mutation showed that significant effects on weight at the birth stage (4.08±015 g.d) and body weight gain until one to three months (295.94±10.19 g.d) respectively. Result of this study showed that this mutation is significantly reduced birth weight significantly. Additionally, as result was revealed, the relationship between weight traits with S3515T/G marker showed that there were significant differences on weight for body weight gain for 3 to 6 months of sheep age (137.91±20.55 g.d). Also, the investigation of relationship between weight traits with S3596C/G marker showed that there were no significant differences between growth parameters about weight gain of one to three months, three to six, six to nine and nine until twelve month.

Discussion

Hajihosseinlo *et al.,* (2015) demonstrated that in the tested Makooei sheep population, significant statistical results were found in all traits of fat-tail measurements for GH and LEP genes. Individuals with the G4, L4 genotype of GH and LEP genes had lower tail length (rump length), fat thickness, and tail width when compared to those of individuals with other genotypes ($P < 0.05$). Also, they showed that individuals with the G5, L5 genotype of GH and LEP genes had superiority of tail length and fat thickness compared to those individuals with other genotypes ($P < 0.05$). Individuals with the G2, L2 genotype of GH and LEP genes had superiority of tail width (rump width) compared individuals with other genotypes ($P < 0.05$). Carro *et al.,* (1997) hypothesized that a dose of leptin that acted to inhibit food intake would be capable of influencing endocrine function. In Moradi Shahrebabak *et al.,* (2016) study, the sequencing results showed the presence of 5 Single nucleotide polymorphism for GH gene in the studied population. No significant associations of the available genotypes in the exon 4 of the ovine GH Gene with carcass traits. In the Cauveri *et al.,* (2014) study the polymorphism in Exon 3 of *LEP* gene in Nilagiri sheep was done by sequencing and genotyping by PCR-RFLP.

Conclusion

The aim of the present study was to determine genetic polymorphism of the leptin gene in selected Lori Bakhtiari sheep breeds mostly used for meat, milk and wool purposes. We are able to generate the basic data in Lori Bakhtiari sheep breeds and open interesting aspects for future selection programs, especially marker assisted selection of animals.

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