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Research Article

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Leptin receptor gene polymorphism and its relationship with functional traits in Baluchi sheep using PCR- SSCP

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ABSTRACT

In this study of 70 Baluchi sheep blood sample collection and DNA extraction was performed by SSCP. The purpose of this study was to evaluate the effect of leptin receptor gene and its relationship with functional traits in Baluchi sheep. The results showed that the haplotype Type I, Type II, Type III mutated haplotype the fourth type of mutation was observed. Weaning weight compared to that between three and four there is no haplotype but the average weaning weight of all of them with a second haplotype statistically different. Seemed to mean sexual relationship with the physiological effects of male and female sex chromosome, especially "sexual hormones this can differ significantly between male and female lambs during the growth of the show. Twin lambs weighing more than two twins, twins and triplets are more, the difference is due to the characteristics of good nutrition and energy and nutrients by the mother during pregnancy and lactation animals. Generally, given that in most seasons sheep pastures are becoming a significant factor in increasing the growth traits not unexpected.

Key words: leptin, extraction of DNA, mutation, sheep, SSCP method

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INTRODUCTION

Identification of polymorphisms in candidate genes and their association with these traits, breeding experts provide the appropriate information. Representative genes for studies, based on the known relationship between physiological and biological processes are selected. Leptin receptor genes within the cell membrane and in the cytoplasm are. Six identical form leptin receptor (Ob-Rf, Ob-Re, Ob-Rd, Ob-Rc, Ob-Rb, Ob-Ra) have been found in various tissues. By a mechanism independent of insulin, leptin, and transmitted to the brain [1, 2].

Leptin receptors are a family of cytokines that signal through the mechanism of message transmission and transcription-activating function [4, 3]. Many advances in molecular genetics and biotechnology were the result of powerful new tools for genetic modification of animals is provided. One of the most useful tools, the DNA markers. Heritable genetic markers and nucleic acid sequence of the DNA sequence between individuals within and among populations shows [4]. Landraces in each country as a strategic national asset that has been preserved and reproduction of this race are of great importance [5].The purpose of this study was to evaluate the effect of leptin receptor gene in Baluchi sheep.

EXPERIMENTAL SECTION

Abbas Abad Mashhad (Iran) station Baluchi sheep population the population studied. A total of 70 blood samples were taken randomly from the population studied. Its purpose was to be seen more different mutations. All blood samples were collected from the jugular vein in the neck and using a vacuum tube containing 5 ml were prepared containing an anticoagulant.

- Extraction of DNA (PCR): DNA was extracted from blood samples were performed using silica gel guanidine thiocyanate. To determine the concentration of DNA, a 1% agarose gel was used.

- Selection of Primers: In this study, leptin receptor gene amplification was used for this purpose, using appropriate software, Oligo arthritis (AO) 7 primers were designed**.** In order to choose the best starter measures such as the ΔG Part 3 and 5 as well as primer dimer and Herpin was desired. The next step primers PIC primer design software to the global site control and the NCBI gene amplification was confirmed.

- The polymerase chain reaction: Polymerase chain reaction in a final volume of 25 micro liters whose composition is as follows: A single tag polymerase enzyme, 200 micro mol of each dNTP, 200 mmol MgCl2, mixture primers (10-20 pmol), DNA (50-100 ng), Standard buffer was used. The polymerase chain reaction with the 35 thermal denaturation step at 95 ° C for 45 seconds, Junction temperature of 58 ° C for 45 seconds, Proliferation at 72 ° C for 45 seconds, Duplicate final temperature of 72 ° C for 8 min. For polymerase chain reaction products were analyzed by 1.5% agarose gel. Gel stained with ethidium bromide (10 mg ml) was performed. To view the digested fragments were obtained in 8% acrylamide gel.

- SSCP method: To perform this procedure 5 microliter of the PCR product was loaded with 10 ml of buffer (Formamid of 95%, 1 mM sodium hydroxide, and 0.25% phenol blue, 0.35% xylene Syanol) the mixture was. The PCR product samples were heated for 15 min at 95 ° C and were immediately put on ice. The samples were placed in a freezer until use within. By 30% at a 1:19 solution of acrylamide gel (6%) was used to prepare the base acrylamide. Samples with a voltage of 55 V for 21 h and vertical electrophoresis were. Silver staining was used to visualize the genotypes.

-Statistical analysis: ANOVA with GML procedures and through SS3 and compared through the least square means were performed using SAS software. To analyze the data using the number of ears, Pedigree information including birth weight, weight at weaning weight of 6 and 12 months, ordered, the data were recorded pedigrees. The effects of sex, type of birth and year of birth herd haplotype using SAS software and survey results were presented in tables.

RESULTS AND DISCUSSION

Mutations in several genes in the range of 92 to 624 observed. Study also found no effect of the deletion mutants was not examined.

The first type haplotype compared with the original sequence with access number DQ 788631 in the NCBI website

Table 1: The first haplotype modified

NCBI/mutun	The number of amino acid bases	Number of genes
A/T	92	10
C/T	94	10
C/G	98	10
A/C	102	10
G/T	103	10
C/A	126	10
C/G	127	10
T/G	128	10
T/A	129	10
G/C	135	10
A/T	142	10
G/T	147	10
G/T	199	10
C/T	205	10
A/T	243	10
T/C	247	10
G/A	397	10
T/A	439	10
T/C	440	10
G/C	583	10
T/A	624	10

Type II haplotype compared with the original sequence with NCBI website access number DQ 788631

Table 2: The second type of modified bases haplotype

NCBI/mutun	The number of amino acid bases	Number of genes
T/C	243	23
G/A	393	23
T/C	419	23
G/C	579	23

The third type haplotype compared with the original sequence with NCBI website access number DQ 788631

Table 3: The third type of modified bases haplotype

NCBI/mutan	The number of amino acid bases	Number of genes
A/T	80	30
C/T	82	30
T/G	83	30
G/T	88	30
C/G	122	30
T/G	123	30
T/A	124	30
G/C	578	30

The fourth type haplotype compared with the original sequence with NCBI website access number DQ 788631

Table 4: The fourth type of modified bases haplotype

NCBI/mutan	The number of amino acid bases	Number of genes
C/T	54	63
A/T	65	63
A/C	70	63
C/T	83	63
C/G	87	63
A/G	89	63
G/C	90	63
G/T	91	63
T/C	98	63
T/C	102	63
G/A	103	63
C/A	126	63
A/G	127	63
A/G	128	63
G/C	132	63
C/T	142	63
C/T	177	63
T/A	194	63
G/A	208	63
T/A	211	63
C/A	220	63
C/T	226	63
A/T	240	63
C/T	256	63
G/T	265	63
G/T	272	63
A/C	273	63
C/T	275	63
C/G	278	63
G/T	282	63
G/A	287	63

Find mutations in gene sequences compared with the genes of the World Bank Find a modified amino acid, making it possible to reproduce. First sequences in the NCBI Web site www.expasy.ir examined and then were Blast genes.

In the first four mutant haplotype that included: Conversion of threonine to alanine and methionine, Lysine to glutamate, Arginine to glycine, Glutamine to histidine, respectively.

The second type of mutant haplotype include: Lysine to glutamate, glutamate to serine, serine to leucine and Glutamine, tryptophan to Arginine, methionine to valine and threonine to glutamate. The second type of mutations observed haplotype that includes: Conversion of glutamate to aspartate, Asparagines to histidine, Arginine to Lysine. The fourth type of mutation and haplotype differences in amino acid was observed.

Haplotype effects on functional traits:
The mean of birth weight was not significantly different between the haplotype.

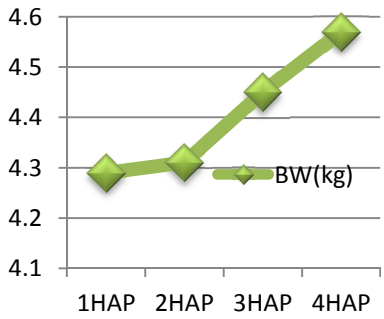

Figure1: Mean of the weight in birth time

Mean weight at weaning through Duncan test showed no significant difference between different haplotypes.

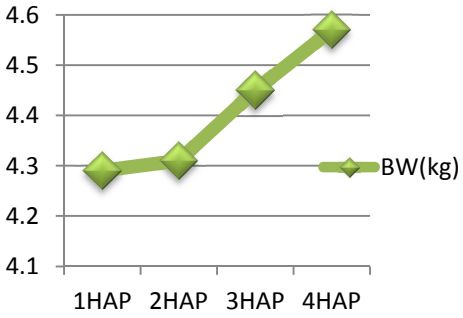

Figure 2: Mean of the weaning weight

The mean weight of six and nine months, there was no significant difference between different Haplotypes.

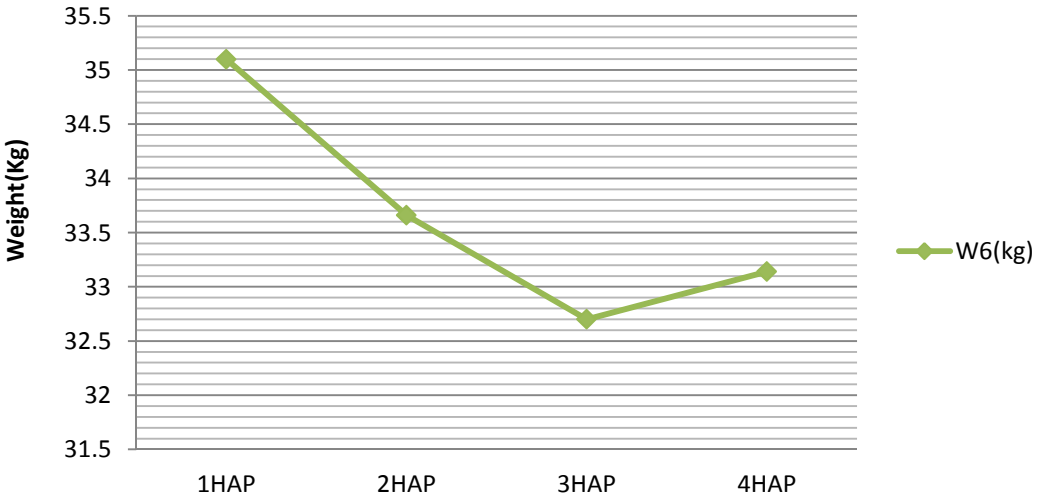

Figure 3: Mean of the weight six months, nine months and twelve months

The essential amino acid threonine to alanine bonded polar and non-polar hydrophobic amino acid that is converted. Threonine deficient energy is reduced was converted to alanine. Alanine is a generator of energy and helps to regulate blood sugar and its deficiency causes the muscles. Lysine is an essential amino acid and reduction in muscle protein synthesis and collagen formation and effective was converted to glutamate. Glutamate is an amino acid that is essential and Structure of nerve cells in the blood and helps. Arginine is an essential amino acid and polar and very game and as a stimulus for the release of growth hormone was converted to glycine. The simplest non-polar amino acid glycine, the smallest and hydrophilic amino acids involved in building red blood cells. Glutamine, non-essential amino acids and polar regulation of acid-base balance in all helps to build ammonium The use of glutamine

by the intestinal cells are was converted to histidine. The amino acid histidine is the root of the game and only a relatively large amount of hemoglobin and Critical role in the metabolic processes of protein. Low level of lysine loss of muscle protein synthesis and the formation of collagen and affects. Lysine, L-carnitine and vitamin C together makes. L-carnitine muscle cells are not able to consume oxygen, was converted to glutamate. Essential amino acids glutamate and substance in human blood, the most abundant free amino acid is. Serine was converted to the amino acid glutamate. Serine compound is involved in the manufacture of fat and protein. Serine was converted to leucine. Tryptophan is Coenzyme causing neurotransmitters and neurotransmitter secretion is converted to the amino acid arginine. Asparagines, which is polar and hydrophilic and helps in DNA synthesis and lead to the removal of ammonia from the body to become histidine. Research (Mokhtarzadeh, 2008) Leptin receptor gene polymorphism was studied in a population of birds native province. Using HaeIII enzyme genotypes BB and AB, AA reported [6]. Zhang et al, 1994 Leptin receptor studied by means of cloning reported that six of the leptin receptor in the form of ISO forms. One of them is the long isoform [7]. In Dridi et al, 2015 research , Leptin gene was cloned and identified that this gene contains three exons separated by two introns are expressed in parts [5]. In another research by (Taouis et al, 1998), Leptin gene mRNA was detected in poultry, about 18 exons coding for leptin receptor gene sequencing was. Furthermore, leptin gene cloned in the expression of leptin in the liver of poultry birds reported that the possibility exists [8]. Schankel and colleagues (2006) found only 2 mutant genotype [9]. Also Komisarek and Dorynek in 2006, 219 Jersey cows were examined and 12 mutations in mutant genotypes were observed [10]. The effect of gender on birth weight, weight of 9 months and 12 months, significant weight, but the weight and weaning weight was 6 months old nonsense. The effect of birth weight on birth weight was significant at 6 and 12 months. But for weaning weight and 9 months is absurd. Type effect on birth weight, weaning weight, weight at 6 and 12 months was significant, but the weight was 9 months old nonsense. Because no significant birth weight, six and nine months, it can be justified, It happened with that type of amino acid mutations in some cases, but not the nature of the physiological changes that alter protein, Or mutations are not so long and in remote areas has occurred. The study showed that there is a mutation in the leptin receptor gene Baluchi sheep that the results of Zhou et al in 2009 that New Zealand was the country's six main race is consistent [11]. In other studies, leptin receptor mRNA expression in adipose tissue of sheep and former pituitary and changes in position in the hypothalamus or limited in terms of nutrition, Show that the full-length leptin receptor in the hypothalamus, pituitary and adipose tissue expressed earlier Finally, the results showed that the self-regulatory mechanisms of leptin in the hypothalamus, depending on the type of diet [10]. In comparison, there was no significant difference between the two herds. Management methods, different climatic and environmental conditions in different herds on body weight and daily weight gain of all ages can have a significant effect, but as the geographic, educational system, method of feeding and herd management in both uniform and random selection caused no significant relationship. This difference appears to be related to the physiological effects of both male and female sex chromosome, especially "sexual hormones can differ significantly between male and female lambs during the growth of the show. The amount of this hormone is different in the two sexes; finally, "the males are heavier than females. A twin lambs weighing more than two twins, twins and triplets are more the difference is due to the characteristics of good nutrition and energy and nutrients by the mother during pregnancy and lactation animals.

CONCLUSION

Generally, given that in most seasons' sheep pastures are, the significance of the birth year of growth traits not unexpected. Because of the amount of annual rainfall, pasture and fodder status, presence or absence of specific diseases and parasites are different.

Acknowledgements
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