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Polymorphism of the prolactin and leptin genes of Awassi sheep

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Summery

Thes research It was erected at the goat and sheep station research at Thi-Qar Governorate, District Shatrah, Agriculture Directorate Thi-Qar. A total of 50 Awassi sheep were used, from 1/11/2024 to 30/6/2025 for one productive season, 3 to 5 years old of age. The genetic work was carriedout in Lab genetic Marshes Research of Thi-Qar University, purpose studying polymorphism in prolactin and leptin genes in Awassi sheep. Results nitrogen base sequence gene prolactin showed a size 156 BP, Absence of any change nucleotide location along the studied region of this gene, thus, the results indicate that all the ewes in our studies with respect to the prolactin gene have a homozygous genotype. Results nitrogenous base sequence leptin gene indicated 260 BP, Change presence in site 119 region studied in the gene, the base nitrogenous in cytosine (C) changed to base nitrogenous (T) thiamine.

Keywords: Polymorphism, prolactin, leptin, genes, Awassi sheep.

Introduction

Great development took place in biology molecular during last twenty years, led to emergence of a type new from genetic marker DNA markers , markers molecular, which was characterized its accuracy in detecting genes responsible for desired traits, molecular markers also provide a technology to conserve plant and animal genetic resources, facilitate the process of biodiversity analysis (Jordana *et al*., 2003).

Prolactin is a milk-producing hormone plays a role at milk production and a lack of prolactin production causes a severe decrease in milk secretion (Knight, 2001).

The prolactin gene It is composed of five exons and four introns, it is on chromosome number 20 in sheep at the site 34,600,253 to

34,608,742 for the reference copy registered under the code NC_001009306.1 on the NCBI Gene Bank website and encodes 229 amino acids of the peptide chain of the prolactin hormone, it is secreted into blood by anterior lobe of pituitary gland , its first goal is to work on stimulating the epithelial cells of mammary gland to produce milk (Senger, 2005).

In recent times, he was there a lot because of the importance of the leptin gene and hormone as a means of biological control, which is related to basic characteristics in raising and improving animals, such as feed intake, the percentage and quality of fat in meat, tenderness and juiciness in meat. (Geary *et al*., 2003).

leptin gene located on fourth chromosome at cattle, goats and sheep, leptin receptor gene in cows is located on chromosome number three, while in humans the leptin gene is located on chromosome number seven. (Perucatti, 2006).

The gene is about 20 kilobases in size and contains three exons separated by two introns, tThe three exons of the leptin gene cover approximately 15 kilobases of the DNA genome, the complete code region is located in the second and third exons and is separated by 2 kilobase introns, part of the second exon codes for the remaining twenty-one amino acids, which was not represented in the mature protein leptin, the structure of the leptin gene, the exon/intron boundaries, and the amino acid sequences in lactose have been studied extensively" (Gong, 1996).

The aims study to know polymorphism prolactin and leptin genes in Awassi .

Methods and Materials

Thes research It was erected at the goat and sheep station research at Thi-Qar Governorate, District Shatrah, Agriculture Directorate Thi-Qar. A total of 50 Awassi sheep from 1/11/2021 to 30/6/2022 for one productive season, 2.5 to 5 years. Data sheep in experiment at collected by knowing ages and types of the animals from animals record, genetic practic also performed in Research Marshes Lab of Thi-Qar University.

The measurement the concentration of the hormone prolactin and leptin, the Korean company Bioassay used the method described in the measurement kit. Then the absorbance was measured using an Elisa Blood device at a wavelength of 450 + 10 nm.

Measuring method

1. The sample Prepared at 25^0c before adding reagents.
2. Add up to 50 µl of standard solution to 2 ml Eppendorf.
3. Then add 40 µl from sample.
4. . Add up to 50 µl of HRP streptavidin.
5. samples cover at a of 37^0C to 60 minutes.
6. . Add up to 50 µl substrate A.

7. washed samples5 times by Wash Bufffer and after washing process, samples are incubated for 1 hour at 37^0c.

8. wavelength device is set to a of 450, and each sample read by optical density .

Extracted DNA of samples blood used a (Kit) supplied by Geneaid mead in Korean, accorded following stages:

1. A 100 m.l from blood and place in test tube 1.5 m.l Eppendorf.
2. Put 100 m.l from solution PBS .
3. Put 10 microliters from Proteinase K about 20 µg/ m.l .
4. Put 10 m.l RNase about 20 µg/ m.l.
5. samples placed in mixed by mixer a vibrating and incubated at 25^0c for 2 minute.
6. Put 100 µl from lysis (GSB) and mixed in mixer about 1 minute.
7. 10 minutes in Incubation at 60°C in a water bath.
8. A solution of 100% absolute ethanol was added, 100 µl, and then the mixer was shaken for one minute.

9.Place 600 ml in a filter tube with a collection tube of the mixture, add it to a filter tube, then place it in a centrifuge at a speed of 14,000 rpm and the filtrate is discarded.

10.Add 200 µl of wash buffer 1 and centrifuge it at 14,000 rpm and then discard the filtrate.

11. Add 300 µL of wash solution 2 at 14,000 rpm for 3 minutes in the centrifuge and then discard the filtrate.

12. Take a new 1.5 ml Eppendorf tube and place a filtration tube in it. Then 50-75 µl of filtration solution was added and centrifuged at 14,000 rpm for a minute and a half.

Primers for the leptin and prolactin genes were prepared by Alpha DNA Company, in the form of a lyophilized powder from two separated primers, each primer placed in a special tube with a label indicating the sequence of the nitrogenous bases. The primers were prepared by adding a specified amount of distilled water, dd water, to start with a concentration of 100 picamoles. This was a stock solution, 10 microliters of it were taken, and then 90 microliters of dd water was added, and the initial concentration was 10 picamoles, the concentration required to perform the PCR. The following table shows the dilution of the starters and the added amounts of distilled water (dd water).:

Table (1): Dilutions of prefixes and added quantities.

Gene	Primer code	Bases No.	GC percent (%)	Dd water Quantities (microliter)	concentration (Bicamol/Macroliter)
Prolactin	PLR F	24	55	1335	100
	PRL R	24	54	1058	100
Leptin	Lep F	19	58	1785	100
	Lep R	20	55	1018	100

Results and discussion

Polymorphism of the prolactin gene in Awassi .

Results of the nitrogen base sequence analysis of the prolactin gene showed a size of 156 base pairs, there was no change in any nucleotide position along the studied region of this gene, thus, the results indicate that all the ewes in our studies with respect to the prolactin gene have a homozygous genotype. Our study was also opposed by Ramos *et al*. (2009) when studying the German Chios sheep breed, where he found genetic formations when studying a piece of the prolactin gene, which was determined by exon 5, where the study was conducted on 247 randomly selected ewes from the German Dorine farm.Nucleotide sequencing was used after DNA isolation, as shown in the following figure.

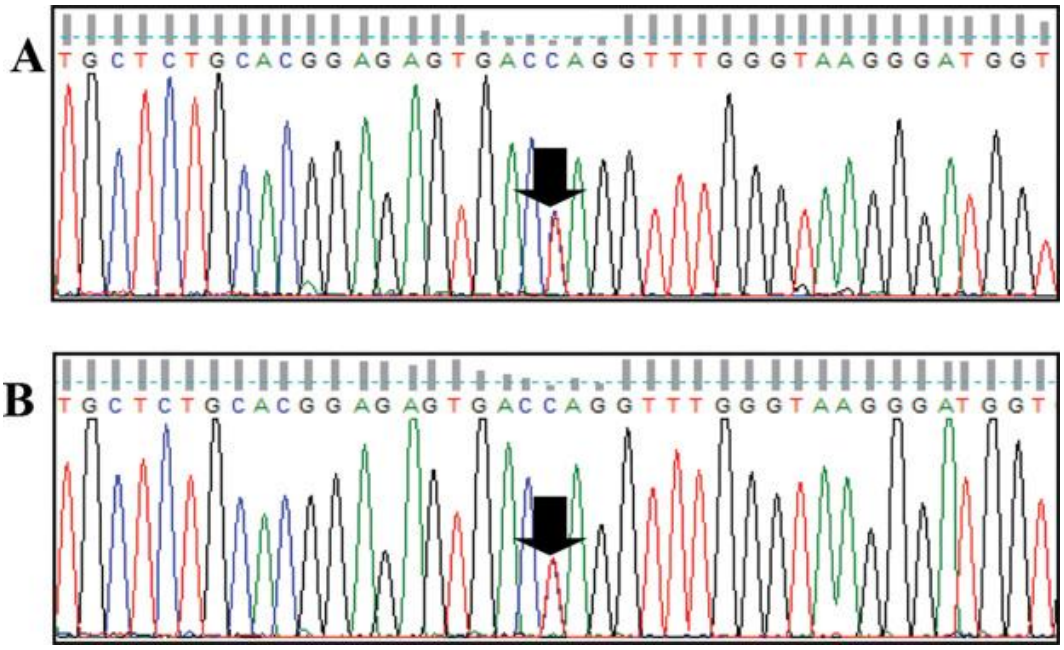

Figure (1) : Polymorphism of prolactin gene in Awassi sheep.

Polymorphism of the leptin gene for Awassi sheep

Results of the sequence analysis nitrogenous base of leptin gene indicated a size of 260 BP, presence of a change in site 119 of the studied region , nitrogenous base of cytosine C was changed to nitrogenous base T . Results showed that the homozygous genotype CC was superior to a larger number of Sheep.

Number was 23 compared to 19 and 8 for the CT and TT genotypes. Gene frequency of genotypes for CC, CT, and TT genotypes was 0.46, 0.38, and 0.16, respectively, while frequency of C and T alleles was 0.65 and 0.35, respectively (Figure 2 and Table 2). , studyAgreed with our Barzkar. et al. (2009), they obtained single nucleotide matches of second intron of A113G SNP and AA leptin using sequencing. To find out genotypes of leptin gene locus, they used the SSCP method, as well as Zhou et al. (2009) studied the third exon of gene leptin from the New Zealand strain and discovered five SSCP genotypes or novel genotypes representing five different sequences. suggest that these sequences represent the alleles of this gene, four SNPs discovered, three of which were produced by changes in amino acids, they emphasized that this discrepancy, may affect leptin activity and function. In the current study, two single nucleotide conformations (SNPs) were found, which differs from other studies. Reason for the difference in results may be due to the difference in the studied strain, and the possibility of mutations that led to a change in the genetic structure of DNA in some European communities.

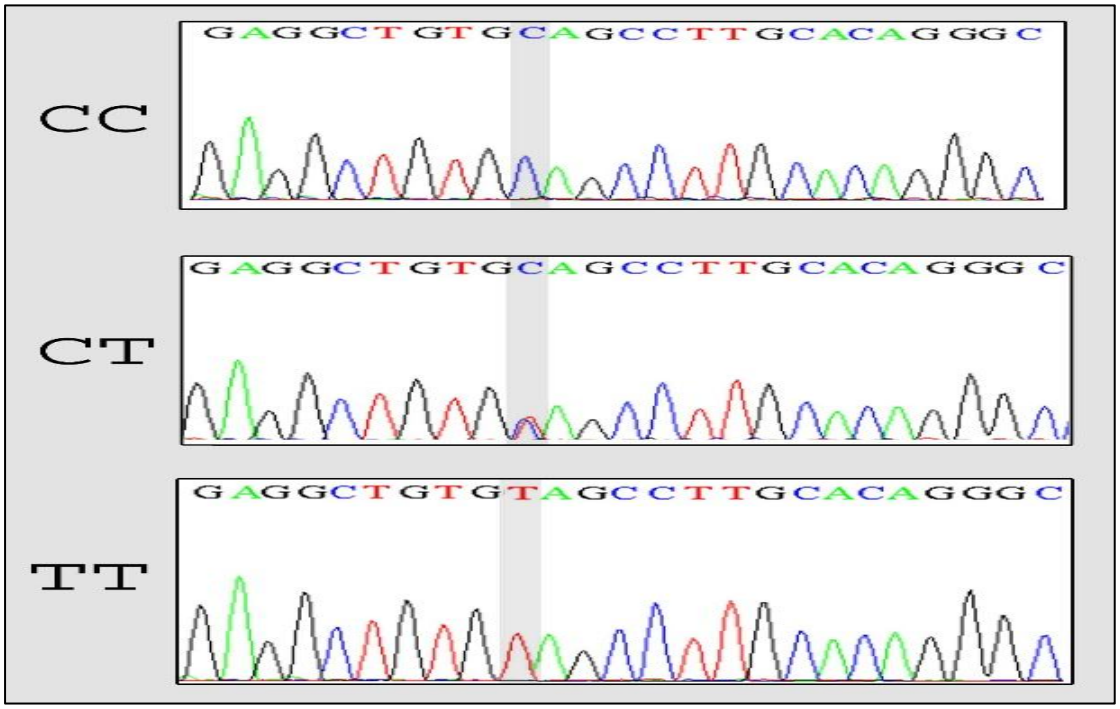

Figure (2.): Mutation (change in the nitrogen bases) of the 119.C>T site of the studied segment of the LEP gene in which the C to T base was changed

Table (2) show the number and frequency of the genotypes of the studied segment of the leptin gene, it shows the presence of three genotypes CC, CT, TT, as for the frequency of genotypes, it showed highly significant differences (P≤0.01), where it was 0.46, 0.38 and 0.16, respectively, where the superiority of individuals with CC . genotype was shown, the number of ewes carrying the CC genotype was 23, the ewes with CT genotype are followed by 19 ewes, followed by the genotype TT is 8 sheep.

Table (2): shows the frequency of the genotypes and the frequency of the C and T alleles of the LEP gene for the variable locus 119.C>T.

Gentype	Animal No.	Genotype frequency	Alleles	Frequency	chi-square value
CC	23	0.46	C	0.65	1.36
CT	19	0.38			
TT	8	0.16	T	0.35	
Total	50	1.00		1.00	

References

Barzehkar R., A. Salehi and F. Mahjoubi 2009. Polymorphism of the ovine leptin gene and its association with growth and carcass traits in three Iranian sheep breeds. Iran J. Biotechnol. 7:241-246.6

Gong, D.W., S. Bi, R.E. Pratley and B.D. Weintraub. 1996. Genomic structure and promoter analysis of the human obese gene. J Biol Chem., 271: 3971-3974.

Jordana, J., P. Alexandrino, A. Beja-Pereira, I. Bessa, J. Canon, Y. Carretero, S. Dunner, D. Laloe, K. Moazami-Goudarzi, A. Sanchez and N. Ferrand. 2003. Genetic structure of eighteen local south European beef cattle breeds by comparative F-statistics analysis J. Anim. Breed. Genet., 120: 73-87.

Knight, C.H. 2001. Overview of prolactin's role in farm animal lactation. Livest. Prod. Sci. 70:87-93.

Ramos, A.M., C.A.P. Matos, P.A. Russo-Almeida, C.M Bettencourt, J. Matos, A. Martins, C. Pinheiro, and R. Figueiredo. 2009. Candidate genes for milk production traits in Portuguese dairy sheep. Small Rumin. Res., 82:117-12 .

Senger, P.L. 2005. The puerperiuman lactation Pathways to and parturition. p 326-345.

Zhou H., J.G.H. Hickford and H. Gong. 2009. Identification of Allelic Polymorphism in the Ovine Leptin Gene. Molecular Biotechnology 41: 22-25.