ARTICLE / INVESTIGACIÓN

The leptin gene's polymorphism and how it relates to the Awassi sheep's physical characteristics

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Abstract: The study was conducted at the sheep and goat research station, at Al-Shatrah District, Thi-Qar Agriculture Directorate, Thi-Qar Governorate, from 1/11/2021 to 30/6/2022 for one production season. A total of 100 animals were used, consisting of 50 parents and 50 lambs, males and females; the ages of the ewes ranged from 3 to 5 years old. The sheep data used in the experiment were collected from the station records. The genetic part was also completed in the Marshes Research Laboratory at the University of Thi-Qar. The study aimed at the genetic polymorphism of the leptin gene and its relationship to the body dimensions of the Awassi sheep. The nitrogenous base sequence analysis of the leptin gene indicated a size of 260 base pairs, with a change in location 119 in the studied area. The homozygous CC genotype was more significant than the number of animals; the frequency of the C and T alleles was 0.65 and 0.35, respectively. There was no significant effect between the three genetic polymorphisms resulting from the mutation at site 119 of the studied plot on the body dimensions of the newborns as well as at weaning.

Key words: Polymorphism, leptin gene, body dimensions, Awassi sheep.

Introduction

The remarkable development in molecular biology and statistics saves the possibilities in selecting a genetic variation and critical genes in genetic improvement[1]. The traditional genetic improvement of farm animals relied on selection for individuals with better phenotypic, as it achieved good economic feasibility in the field of genetic modification; scientific development has also helped in knowing the work of the genome, which made it possible to develop more accurate, less time and costly selection programs, as economic traits are under the control of several genetic loci, which was known as the location of quantitative traits, by selecting these linked sites, the phenotypic variance of the characteristics to be improved can be predicted in a short time, and selection programs can be prepared based on it[2-4].

The leptin gene is located on the fourth chromosome in cattle, sheep and goats; the leptin receptor gene of the bovine family is located on the third chromosome; in humans, the leptin gene is located on the seventh chromosome[5].

Growth in newborns reflects the ability to produce meat and milk in the future; body weights at different stages of life are also considered a guide or indicator of growth, the possibility of benefit in predicting the production of milk after childbirth, as well as the importance of growth traits as an indicator or as a criterion for the evaluation of the herd, starting early by selecting the distinct ones[6].

The concept of the physical structure of sheep is also considered biological; it is the result of the outward forms of the animal's body and the characteristics of its internal organs; the physical structure affects the appearance of the animal, its production, and its ability to lead a productive life, the study of the physical structure aims to clarify the relationship between the structure of the body and some of its parts[7].

Body weight and body dimension can be used as a guide in the future breeding and reproductive efficiency of animals[8,9]. Many factors affect the presence of a significant variation in the sheep herd for these measurements, including the genetic structures carried by the animal, which determines the shape of the animal and its measures, sex, type of birth and the environmental conditions in which the herd lives[10]. Recently, researchers have focused on special measurements in the animal body, which was 67 as an indication that their bodies are full of meat by fattening with successive feeding periods, as well as their economic importance[6].

The current study aims to show the polymorphism of the leptin gene and its relationship to the body dimensions of Awassi sheep.

Materials and methods

The study was conducted at the sheep and goat research station, at Al-Shatrah District, Thi-Qar Agriculture Directorate, Thi-Qar Governorate, from 1/11/2021 to 30/6/2022 for one production season. A total of 100 animals were used, consisting of 50 parents and 50 lambs, males and females; the ages of the ewes ranged from 3 to 5 years old. The sheep data used in the experiment were collected from the station records. The genetic part was also completed in the Marshes Research Laboratory at the University of Thi-Qar. The study aimed at the polymorphism of the leptin gene and its relationship to the body dimensions of Awassi sheep.

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DNA extraction

DNA was extracted from sheep blood samples using a measuring kit (Kit) supplied by the Korean Geneaid Company, according to the following steps:

1. Take 100 microliters of blood and place it in a 1.5ml Eppendorf tube.
2. Add 100 µl of PBS solution (Phosphate buffered saline).
3. Add 10 µl of Proteinase K at a concentration of (20 mg/ml).
4. Add 10 microliters of RNase at a concentration of (20 mg/ml).
5. The samples were mixed using a vibrating mixer (Vortex) and incubated at room temperature for two minutes.
6. Add 100 µl of Genomic lysis / Binding buffer (GSB) and shake the mixer device for one minute.
7. Incubation for 10 minutes in a water bath at 60°C.
8. 100 µl of 100% Ethanol Absolute was added and shaken with the mixer for one minute.
9. Take a filter tube with the collection tube and add 600 microliters of the mixture, put it in the filter tube, and then put it in a centrifuge at 14000 rpm, and the filtrate was disposed of.
10. 200 µl of Wash Buffer1 solution was added and centrifuged at 14,000 rpm, and the filtrate was discarded.
11. 300 µl of Wash Buffer 2 was added and centrifuged at 14,000 rpm for three minutes, and the filtrate was discarded.
12. Take a new 1.5ml Eppendorf tube and place the filter tube in it, then add 50-75 µl of Elution Buffer and centrifuge at 14000 rpm for 1.5 minutes.

Primers processing

The primers for the prolactin and leptin genes were prepared by Alpha DNA, in the form of lyophilized powder, from separate primers, each primer was placed in a unique tube with a label showing the sequence of nitrogen bases, and the primers were prepared by adding a specific amount of distilled water add water to become the initiator with a concentration of 100 Picamol, and this is considered a Stock Solution, 10 µl was taken from it, and 90 µl of dd water was added again, so that the concentration of the primers was 10 pcm, which is the concentration required to perform the PCR reaction Table 1.

Migration of PCR product samples

Agarose gel was prepared with the same previous steps, by which the DNA material of the samples was removed for each concentration of the prepared agarose; for migrate PCR product samples, 1.5%, where 0.45 g of agarose was added to 30 ml of 1X. TAE solution, 5 µL of PCR product was loaded with 2 µL of Dilute Diamond™ Nucleic Acid Dye into each pit on the agarose gel, use a marked hole to load 5 µl of DNA Ladder with the dye, setting the relay program with 70V and 85mA for 45 minutes, after the migration process of the PCR product, images were taken using a UV Gel Documentation device.

Studied traits

Measurement of body dimensions for newborns

Measurements of the body dimensions of the newborn were taken every two weeks during the experiment period by taking body weights at birth and weaning and body dimensions at these two ages as in the following method:

1. Height of the body at the front: The distance from the point of contact of the neck with the body to the ground (which represents the height of the body at the front) was measured using a ruler.
2. Height of the body at the back: The distance from the endpoint of the back to the floor (which represents the body's height at the back) was measured using a ruler.
3. Breast Circumference: This area was measured by wrapping the inserted fabric tape around the breast area (just behind the front legs) with tapered tape.
4. Abdominal Circumference: This area was measured by wrapping the inserted tape around the abdomen (just in front of the hind legs).
5. Minimum and maximum temperature: Where the temperatures were taken through mercury lamps hung in the animal pens, where the lowest degrees were taken at six o'clock in the morning and the maximum at three o'clock in the evening.
6. Heat Tolerance Calculating (HTC): for newborns, according to the following equation (Reece, 2009),

HTC=100 –[18 (RT -39.1)]

Since:

RT = average rectal temperature in the morning and afternoon

39.1 = normal rectal temperature

18 = fixed

7- Calculate the body mass index using the following equation:

$$\text{Body Mass Index} = \frac{\text{animal weight}}{\text{animal length}}$$

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

Table 1. Dilutions of the prefixes and added quantities.

F: 5' - -3' CGCAAGGTCCAGGATGACACC R : 5' - -3' GTCTGGGAGGGAGGAGAGTGA	LPE

Table 2. The primer sequence was used in the study. Gene Sequencing Forward.

Gen	Stages	Temperature (C◦)	Time (Minutes)	Cycle number
Leptin	Initial metamorphosis	95	5	1
	Metamorphosis	95	0.30	35
	Adhesion	62	0.45	
	Elongation	72	0.30	
	Final elongation	72	10	1

Table 3. PCR product migration preparation process.

Results

Electrophoresis results showed that using 1% agarose gel, the samples were migrated by 5 µL of the DNA sample and 2µL of loading day in 1% agarose gel, contains Diamond™ Nucleic Acid Day tincture prepared by Promeca and diluted by TE, dilute 1 microliter of dye to 100 microliters of TE solution, mix the mixture well using a micropipette on a plastic strip (Laboratory Film), after adjusting the voltage, the agarose gel was examined using a UV Gel Documentation device, migration images were taken using the purpose-built camera Fig. 1.

The results of the nitrogenous base sequence analysis of the leptin gene indicated size of 260 base pairs, the presence of a change in site 119 of the studied region of this gene, the nitrogenous base of cytosine C was changed to the nitrogenous base T of thiamine. The results showed that the homozygous genotype CC was superior to a more significant number of animals, 23, compared to 19 and 8 for CT and TT genotypes, respectively. The frequency of genotypes for CC, CT and TT genotypes recorded as 0.46, 0.38 and 0.16, respectively; for the frequency of C and T alleles, they were 0.65 and 0.35, respectively Table 4.

The relationship between the genotypes of the studied segment of the leptin gene on the body dimensions of newborns

Table 5 indicates that there was no significant effect among CC, CT and TT genotypes; the result of this mutation is at site 119 of the studied segment of the leptin gene, with both body length, front height, butt height, breast circumference and abdominal circumference for newborns, the results for the genotypes CC, CT and TT were as follows; body length 35.65,36.55 and 36.00, and for the front height 35.95,34.05 and 33.57, and rear height 36.91,34.90 and 35.28, and breast circumference 38.21,38.25 and 39.42, as well as for abdominal circumference 37.56, 37.70 and 36.85, respectively.

The relationship between the genotypes of the studied segment of the leptin gene on body dimensions at weaning

Table 6 indicated no significant differences among CC, CT and TT. In animal genotypes, the result of this mutation is at site 119 of the studied segment of the leptin gene, with the body length, breast circumference and abdominal circumference at weaning; the results for body length were 52.95,35.50 and 53.28, breast circumference 58.34, 57.90 and 56.85, and abdominal circumference 56.73, 56.15 and 56.57, respectively.

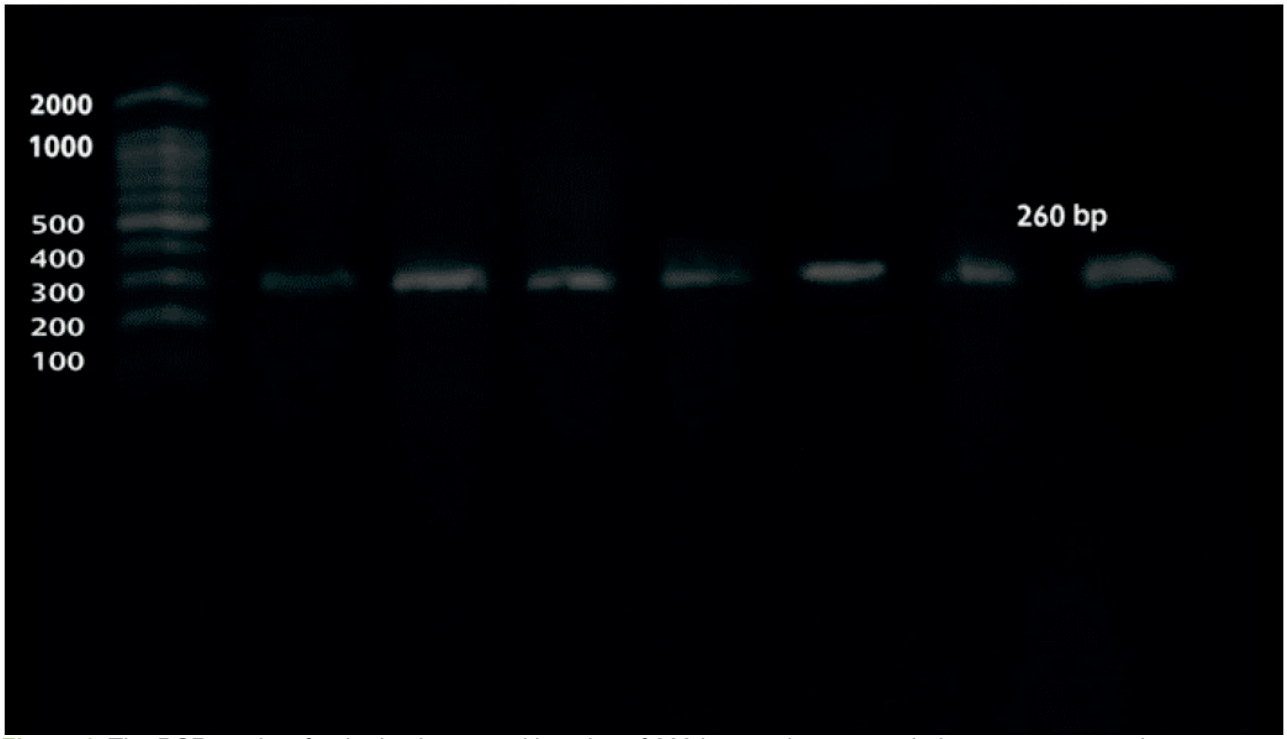

Figure 1. The PCR product for the leptin gene with a size of 260 base pairs was carried on an agarose gel at a concentration of 1.5%.

Genotype	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	

Table 4. 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.

Genotype	Animal No.	Body length	Front height	Rear height	Breast Circ.	Abdominal Circ.
CC	23	35.65±0.45	35.95±1.34	36.91±1.34	38.21±0.46	37.56±0.37
CT	19	36.55±0.48	34.05±0.51	34.90±0.89	38.25±0.53	37.70±0.44
TT	8	36.00±0.57	33.57±0.92	35.28±1.89	39.42±0.52	36.85±0.82
Sig.		NS	NS	NS	NS	NS

Table 5. The relationship between the genotypes of the studied segment of the leptin gene in body dimensions at birth.

The results in Table 6 also showed significant differences (P≤0.05) between animals carrying the three genotypes CC, CT and TT, resulting from this mutation at site 119 of the studied segment of the leptin gene with high fore and hindquarters at weaning. The results were as follows: 57.60, 55.70 and 59.00, and 59.82, 58.40 and 61.28, respectively; the average values were 4.40, 4.01 and 3.89 kg, respectively, in Awassi sheep, however, the average body length was 70.85 and 73.27 cm, average front height 51.74 and 53.17 cm, the average height of the back is 54.00 and 55.66 cm, average breast circumference 90.82 and 93.20, the average hip circumference was 94.55 and 97.41 cm, for single and twin births, respectively.

Discussion

Electrophoresis results showed that using 1% agarose gel, the samples were migrated by 5 µL of DNA sample and 2µL of loading day in 1% agarose gel, contains Diamond™ Nucleic Acid Day tincture prepared by Promeca and diluted by TE, dilute 1 microliter of dye to 100 microliters of TE solution, mix the mixture well using a micropipette on a plastic strip (Laboratory Film), After adjusting the voltage, the agarose gel was examined using a UV Gel Documentation device, migration images were taken using the purpose-built camera[11], that they obtained the single nucleotide conformations of the second intron of the A113G SNP and AA leptin gene using sequencing. Moreover, these results were in agreement with what was obtained[12,13]. While our study does not agree with (14) in Awassi sheep, the values were the most obtained in our study, and the reason was due to the education system and mothers' nutrition. (15) explained that the type of birth has an effect on body measurements or dimensions[16,17].

Conclusions

The results of the nitrogenous base sequence analysis

Genotype	Animal No.	Body length	Front height	Rear height	Breast Circ.	Abdominal Circ.
CC	23	52.95±0.62	57.60±0.63 AB	59.82±0.36 AB	58.34±0.78	56.73±0.81
CT	19	35.50±0.71	55.70±0.39 B	58.40±0.48 B	57.90±0.54	56.15±0.65
TT	8	53.28±1.30	59.00±1.34 A	61.28±0.42 A	56.85±1.12	56.57±1.10
Sig.		NS	0.05	0.05	NS	NS

Table 6. The relationship between the genotypes of the studied segment of the leptin gene in body dimensions at weaning.

of the leptin gene indicated a size of 260 base pairs, with a change in location 119 in the studied area. The homozygous CC genotype was greater than the number of animals, the frequency of the C and T alleles was 0.65 and 0.35, respectively. There was no significant effect between the three genetic polymorphisms resulting from the mutation at site 119 of the studied plot on the body dimensions of the newborns as well as at weaning.

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