# RELATIONSHIP OF THE LEPTIN GENE POLYMORPHISM ON THE PRODUCTION PERFORMANCE IN LOCAL AWASSI LAMBS

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**ABSTRAC**
**This study was conducted at the Animal Farm of the College of Agricultural Engineering Sciences, University of Baghdad during the period from 22/1/2022 to 2/4/2022. This study was conducted to investigate the effect of leptin gene polymorphism on the weight gain, digestibility and rumen fermentations of Awassi lambs. Forty Awassi lambs were selected at age 4-5 months, with an initial weight mean of 25.4 kg, and distributed randomly in individual pens. The results showed that there were three variations in SNP (G>A), GG, GA and AA it was 66.67, 30.56 and 2.7%, respectively, and the differences between them were highly significant (P≤0.01). No significant differences of leptin gene polymorphism were noticed on the percentage of volatile fatty acids (acetic, propionic and butyric) as well as on the percentage of ammonia nitrogen at all times (0,3 and 6 hours). There were highly significant differences (P≤0.01) in pH of the rumen fluid in GG variations at the times of 0 and 6 hours compared with GA it was 6.78 ± 0.03 and 6.76 ± 0.02, respectively. The results also showed that there were no significant differences in the leptin gene polymorphism on digestibility and weight gain. It can be concluded that the study of genetic variations of leptin gene had no effect on the performance of Awassi lambs.**

**Keywords; weight gain , digestibility , rumen fermentations**



## علاقة تعدد المظاهر الوراثية لجين اللبتين مع الأداء الانتاجي للحملان العواسية المحلية

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**المستخلص**
اجريت هذه الدراسة في الحقل الحيواني التابع لكلية علوم الهندسة الزراعية / جامعة بغداد للفترة من 2022/1/22 الى 2022/4/2 . هدفت هذه الدراسة بيان تأثير التغاير الوراثي لجين اللبتين على معدل الزيادة الوزنية ومعامل الهضم وتخمرات الكرش للحملان العواسي. استخدمت في هذه التجربة 40 حملا ذكرا من الأغنام العواسي بعمر 4-5أشهر وبوزن ابتدائي 25.4 كغم، تم توزيعها عشوائياً في حظائر منفردة. اظهرت النتائج وجود ثلاث طرز وراثية للتغاير (G>A) وهي GG و GA و AA وكانت 66.67 و 30.56 و 2.7% على التوالي وكانت الاختلافات بينهم عالية المعنوية (P≤0.01), حيث اظهرت النتائج عدم وجود فروق معنوية في تأثير التغاير لجين اللبتين في نسبه الاحماض الدهنية الطيارة (الاسيتيك و البروبيونيك و البيوتيريك) وكذلك في نسبة نيتروجين الامونيا و لجميع الاوقات (6,3,0 ساعة).بينما لوحظ وجود فروق عالية المعنوية (p≤0.01) في الاس الهيدروجيني (pH) في سائل الكرش لصالح التغاير GG في الاوقات 0 و6 ساعة, اذ بلغت 6.78و 6.76على التوالي . كذلك بينت النتائج عدم وجود فروق معنوية للتغاير الوراثي لجين leptin في معامل هضم العناصر الغذائية ومعدل الزيادة الوزنية . يمكن الاستنتاج بان دراسة التغايرات الوراثية لجين اللبتين ليس لها تأثير في اداء الحملان العواسي.

الكلمات المفتاحية: الزيادة الوزنية، معامل الهضم، تخمرات الكرش.



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## INTRODUCTION

Sheep are one of the most economic important local animals. Sheep were initially raised by following the method of natural selection or by relying on their productivity during the period of their rearing in the field, but this approved system slowed down the productivity of sheep and the possibility of benefiting from Its economic characteristics to improve the nutritional and productive efficiency of the animal through genetic improvement techniques, many genes and their genetic variants have been identified that have an association with phenotypic traits and have economic importance (12,2). A protein hormone called leptin is released by white adipose tissue. It has a significant impact on energy metabolism and may have an impact on food intake, body energy homeostasis, and nutrient partitioning amongst tissues(5). Leptin also regulates lactogenesis, which is another benefit (11,19). Growth is an important economic trait in sheep (1,26,31). Evidence indicates a significant diversity in the leptin gene in sheep, with clear associations with growth traits and carcass characteristics (2, 32, 23, 28). The single nucleotide polymorphism (SNP) of the leptin gene in intron 2 was significantly associated with birth weight and average daily weight gain in Saanen goats (36). These results demonstrate that LEPSau3AL can be used as an effective genetic marker in growth improvement programs in goats (6, 7, 27, 37). Selecting genes is an important and effective method for genetic improvement of flocks (3, 23) and among these genes is leptin gene, as it is associated with a number of productive traits that are related to nutrition especially in sheep, such as gender, puberty, reproduction and milk production. It also has a fundamental role in growth and feed conversion efficiency (1). Other studies have shown that the leptin gene has a regulatory function in body weight, the amount of feed intake and growth in sheep (4,13, 21), In a study, it was found that the leptin gene influences meat quality and fat content, which affects carcass characteristics in both cows and bulls (14, 17, 25, 30, 38, 42). The results of the study also showed that the birth weight, weaning weight, and weight gain rate were significantly ($P \leq 0.05$) related to the maternal genotype, and the best performance was in lambs from mothers carrying two genetic combinations, AA and AB. Therefore, the leptin gene is a promising genetic indicator that can be adopted in genetic selection programs for local sheep (16, 33, 39). The objective of this study was done to determine the relationship of leptin gene polymorphism with weight gain, digestibility and rumen fermentations of local Awassi lambs.

## MATERIALS AND METHODS

This study was conducted at the College of Agricultural Engineering Sciences, Animal Production Department, University of Baghdad for the period from 22/1/2022 to 2/4/2022. Forty Awassi ram lambs were used to detect the leptin gene polymorphism and its association with digestibility, daily gain, ,live weight gain and rumen fermentation in local Awassi lambs. Five milliliters of blood were collected via jugular venipuncture in a collection tube supplemented with (K2 EDTA) anticoagulant, and transmitted in a cool box to the Laboratory of Agricultural Molecular Biology at the College of Agricultural Engineering Sciences, University of Baghdad for cryopreservation under -4 °C, then DNA extraction from blood was don by using a special kit of extraction of DNA .The genomic DNA's integrity was assessed using electrophoresis on agarose gels. The leptin gene polymerase chain reaction (PCR) technique was focused on the major polymorphism observed exon 3 of the leptin gene. The 627 bp segment Gene, while the volume of whole genome for leptin gene was 443534bp observed in sheep. Genomic DNA was amplified using specific primers

F:5'AGAAGTAAGGGTCCAGGAAG3

R:5'CTGAGAGGAGCAAGAGAGA3'

Annealing temperature was started at 58ºC and ending at 48ºC (Touchdown PCR) from Bayer company at 30 cycle for 30 sec. The primer sequence as follows (41). After the end of the PCR-program, the samples were sent to the Republic of North Korea (Nappo Company) for DNA Sequencing. The data were analyzed statistically using Statistical Analysis System (SAS) (35). To study the effect of leptin gene Chi-square test ($\chi 2$) was used to compare the percentages of genotypes distribution for the leptin gene in the sheep sample . Allelic frequency was calculated according to the Falconer and Mackay (18) equation for Leptin gene. As for the chemical analysis of (the concentrate, the roughage, and the waste for the digestion experiment), the dry matter, organic matter, crude protein, crude fiber, ether extract, and nitrogen-free extract were estimated in the postgraduate nutrition laboratory of the Animal Production Department in the College of Agricultural Engineering Sciences(8). Rumen fermentation and volatile fatty acid concentrations were measured after thawing the frozen rumen fluid, filtering it, and then conducting the necessary analyses in the laboratories of the Ministry of Science and Technology / Environment and Water Department using a chromatography device (40). To study the effect of LEPTIN gene polymorphism on the reproduction performance (the first mathematical model) in the studied traits Significant differences between averages by Duncan (15) multiple range test with the application of least square means.

The statistical model was as follows:

$Yij= \mu + Ti + eij$

Where:

Yijk = The observed value,

$\mu$ = Overall mean

Ti=The effect of leptin gene polymorphism

eij= Error term

$$PA = \frac{2 * \text{No. of Homozygous} + 1 * \text{No. of Heterozygous}}{2 * \text{Total number of samples}}$$

First allele replication: PA Since: P + q = 1, then the frequency of the second allele is: Qb

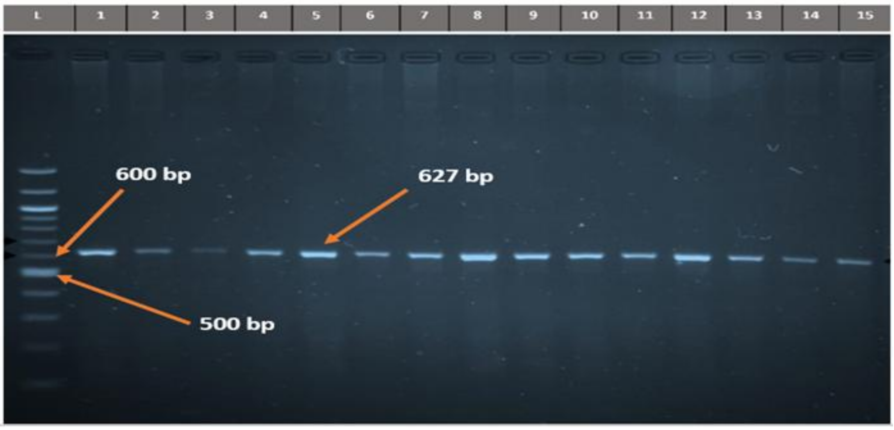


**Figure 1.The electrophoresis of PCR for the Leptin gene ,agarose concentration 1.5%,5volt/cm for1.5hour,ladder(100-1000)bp,lanes1-1,5represent samples with sizes of approximately 627bp**

## RESULTS AND DISCUSSION

### PCR amplification of exon 3 for leptin gene

PCR amplification of the target gene genomic DNA. The results showed the amplification of a fragment size 627 bp of the Leptin gene The PCR products were separated successfully on 1.5% agarose gel with the DNA ladder (100 bp). The samples were amplified successfully by pcR reaction , and a single band stained with Ethidium Bromide was obtained, as shown in the Figure (1). The gene polymorphism, its percentages, and the allelic frequency of the Leptin gene are shown in Table (1). Three polymorphism appeared and the percentages of GG,GA,AAwere 66.67,30.56 and2.77% respectively. Calculater of allele frequency of LEPTIN gene according of Hardy Weinberg´snequilibrium(18).

**The sequences of the nitrogenous bases of the leptin gene:** The nitrogen base sequencing technique was used, and the results revealed that the studied segment of the leptin gene (627base pairs) had in the target coding region of the leptin gene, as three polymorphism appeared in this variation (G>ASNP) which are GG,GA and AA,(18) as shown in Figure (2).

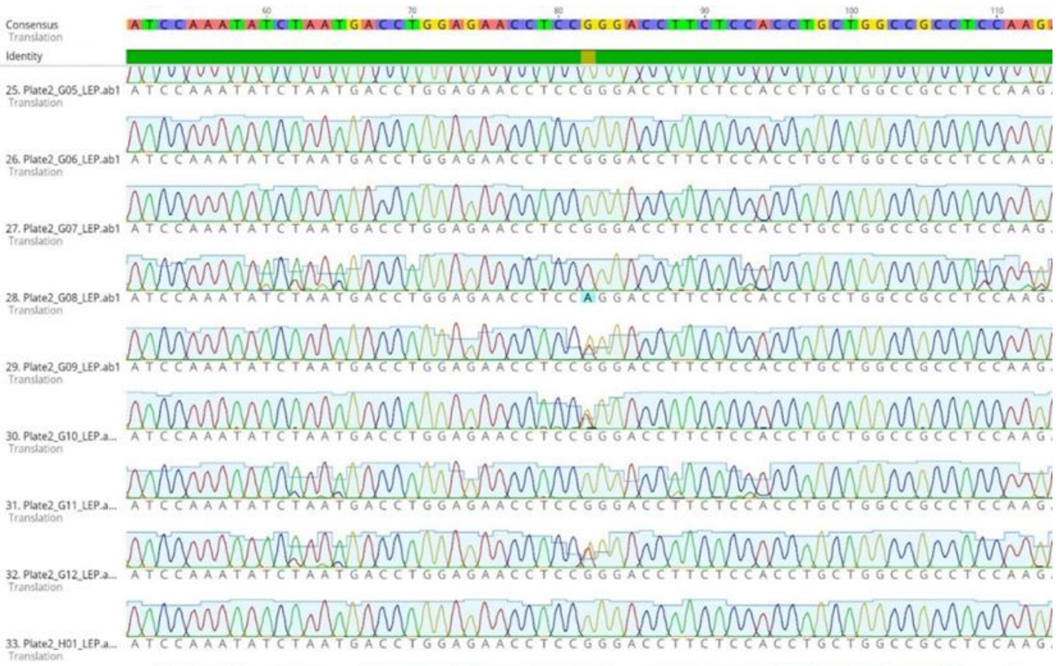

**Figure 2. The sequences of the nitrogenous bases of the LEPTIN gene**

**Table 1. Distribution and allele frequency of the leptin gene G >A SNP polymorphism in a number of Awassi lambs**

| Leptin gene /G >A SNP Polymorphism | Number | Percentage (%) |
|---|---|---|
| GG | 24 | 66.67 |
| GA | 11 | 30.56 |
| AA | 1 | 2.77 |
| Total | 36 | %100 |
| Chi-Square ($\chi$ 2 ) | p-Value | 0.84 |
| Allele | | Frequency |
| G | | 0.82 |
| A | | 0.18 |
| ($P\leq0.01$)** | | |

Table 2 shows the effect of the Leptin gene polymorphism on the rumen fermentations of lambs. The results show that there were no significant differences between the genotypes and the rumen fluid characteristics at different times (0, 3, 6 hours), but there was significant effect of the variants of the Leptin gene on rumen PH at 0 and 6 hours for the same genotype GA, and the ratios were 6.59 ± 0.03 at 0 hr. and 6.61 ± 0.03 at 6 hr. However, it is within the normal limits of the ruminal pH in sheep is between 6.4 and 6.8. Ruminal pH values less than 5.5 or greater than 7.0 are considered abnormal; ruminal pH of less than 5.5 is defined as subacute rumen acidosis (24). Awassi lambs were chosen to evaluate the effects of leptin gene polymorphisms on rumen fermentations and ammonia nitrogen ($NH_3$-N). The results of the current investigation indicated that volatile fatty acids have no apparent effects. This might be a result of a similar diet with the same quantity of protein and energy for all animals; therefore, the may have prevented major variations in feed intake (29). This outcome was linked to the same study's no significant differences on weight gain and digestibility. The Leptin gene *P*olymorphism did not have any significant effects on dry matter intake (9,34). While polymorphisms of the leptin gene were associated with significant differences among genotypes in feed intake in cattle (10).

**Table 2. Association of the leptin gene G>A SNP polymorphism with rumen fermentation**

| Leptin gene SNP (G>A) polymorphism | Means ± SE | | | | |
|---|---|---|---|---|---|
| | PH | $NH_3$-N Mg/dl | Acetic Acid | Propionic acid | Butyric acid |
| **0 hr.** | | | | | |
| GG | 6.78±0.03a | 13.77±1.48a | 11.94±0.10a | 5.90±0.17a | 3.95±0.19a |
| GA | 6.59±0.03b | 14.59±1.21a | 11.65±0.14a | 5.37±0.18a | 3.41±0.28a |
| Level of significance | P≤0.01 | NS | NS | NS | NS |
| **3 hr.** | | | | | |
| GG | 6.63±0.05a | 11.76±1.09a | 16.35±0.42a | 8.68±0.32a | 6.90±0.18a |
| GA | 6.45±0.03a | 13.62±1.11a | 15.09±0.82a | 7.65±0.49a | 6.37±0.03a |
| Level of significance | NS | NS | NS | NS | NS |
| **6 hr.** | | | | | |
| GG | 6.76±0.02a | 11.23±1.14a | 15.26±0.37a | 8.03±0.31a | 6.35±0.21a |
| GA | 6.61±0.03b | 13.35±1.04a | 13.97±0.5a | 7.15±0.54a | 6.09±0.22a |
| Level of significance | P≤0.01 | NS | NS | NS | NS |

Means with different letters in the same column differed significantly, NS: Non-Significant.

Table 3 shows the effect of genotypes the Leptin gene on the digestibility of different nutrients. The results showed that there were no significant differences among the genotypes and the traits studied. The results of this study disagreed with the findings of (21) who mentioned that the Leptin gene is one of the genes that is associated with growth traits.

**Table 3. Association of the leptin gene /G>A SNP polymorphism with digestibility (%)and nutritive ratio**

| Leptin gene SNP (G>A) polymorphism | Means ± SE | | | | | | |
|---|---|---|---|---|---|---|---|
| | Dry matter % | Organic matter % | Crude protein % | Crude fiber % | Ether extract % | Nitrogen free extract % | Nutritive ratio % |
| GG | 67.36±0.36 | 77.88±0.66 | 75.39±0.98 | 57.63±0.64 | 66.46±0.68 | 66.97±0.61 | 54.15±0.93 |
| GA | 66.11±1.41 | 78.30±0.61 | 74.77±1.41 | 55.78±0.65 | 65.52±1.15 | 65.54±1.44 | 52.37±1.13 |
| Level of significance | NS | NS | NS | NS | NS | NS | NS |

Means with different letters in the same column differed significantly, NS: Non-Significant.

Table (4) shows the effect of the genetic variation of the Leptin gene on the rate of weight gain, but there were no significant differences between the different genotypes and the trait studied. Leptin has a variety of purposes, including controlling appetite, body weight, and glucose homeostasis(13)**.** In many sheep breeds, the LEP gene has been associated with growth (1) . In conclusion, the results show a positive influence of GG SNP Polymorphism on pH of Rumen liquor and without effect on other studied traits. The results of the current study differed from previous studies due to the different conditions of the experiment and the animals used in the experiment, as well as the different environmental conditions of the experiment from previous stud

**Table 4. Association of Leptin gene /G>A SNP Polymorphism with initial weight, final weight, total gain, total intake and feed conversion ratio**

| Leptin gene SNP (G>A) polymorphism | Means ± SE | | | | |
|---|---|---|---|---|---|
| | Initial weight (kg) | Final weight (kg) | Total gain (kg) | Total intake (kg) | Feed conversion ratio % |
| GG | 25.26±0.77 | 34.63±.76 | 9.36±0.63 | 71.83±1.67 | 8.11±0.42 |
| GA | 25.75±1,29 | 34.25±1.31 | 8.50±0.62 | 70.37±3.51 | 8.53±0.56 |
| Level of significance | NS | NS | NS | NS | NS |

NS: Non-Significant.

## Conclusion

The results showed the presence of one SNPS for each of the Leptin gene (G>82 A) and (G<292 A) in the blood sample taken from the Awassi male lambs used in the study. Based on the results obtained, there is no significant effect between the genetic compositions of the Leptin gene and the studied traits, except for PH.

**CONFLICT OF INTEREST**

The authors declare that they have no conflicts of interest.

**DECLARATION OF FUND**

The authors declare that they have not received a fund.

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