# RELATIONSHIP OF THE *FASN* GENE POLYMORPHISM WITH MILK PRODUCTION AND ITS COMPONENTS IN LOCAL AWASSI SHEEP

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## ABSTRACT

**This study was conducted to detecting the FASN gene polymorphism and its relationship to daily milk yield (DMY), lactation period, and milk composition in addition to polymorphism distribution and allele frequency in 52 Awassi ewes and their lambs. It was carried out from 5/1/2022 to 30/10/2022 at the Sheep Farm of the Al-Fayhaa station in the Jableh sub-district / Al-Musaib project (55 km south of Baghdad), in addition to the Biotechnology Laboratory at the College of Agricultural Engineering Sciences/University of Baghdad. Three polymorphism appeared in this variant (A>G SNP), which are AA, AG, and GG, with the percentages of 63.46, 32.69, and 3.85%, respectively. The variation among them were highly significant (P≤0.01) with a frequency of 0.70 and 0.30 for alleles G and A, respectively. The protein content of Awassi sheep's milk differ significantly (P≤0.05) at rate 5.17% for ewes with GG polymorphism. In conclusion, FASN gene can be used to develop strategies for genetic improvement of sheep, and expanding the study to a larger sample and multiple sites and study the types of fatty acids in milk, as well as finding the interaction between two SNPs, would give more accurate results and determine the best method for managing and improving sheep flocks.**

**Keywords: Daily milk yield, Lactation period, milk composition.**



# علاقة المظاهر الوراثية لجين FASN بإنتاج الحليب ومكوناته في الأغنام العواسي المحلية

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## المستخلص

أجريت الدراسة بهدف الكشف عن تعدد الأشكال لجين FASN وعلاقته بإنتاج الحليب اليومي، طول موسم الحليب ومكونات الحليب، فضلاً عن نسب توزيع المظاهر الوراثية والتكرار الاليلي للجين في 52 من النعاج العواسي وحملانها في الفترة من 2022/1/5 إلى 2022/10/30 في مزرعة الأغنام التابعة لمحطة الفيحاء بناحية جبلة / مشروع المسيب (55 كم جنوب بغداد) بالإضافة إلى مختبر التكنولوجيا الحيوية في كلية علوم الهندسة الزراعية / جامعة بغداد. ظهرت ثلاثة مظاهر وراثية في هذا التغاير (A> G SNP)، وهيAA و AG و GG، وان الفروق بينها عالية المعنوية (P≤0.01) وبتكرار 0.70 و 0.30 للاليلين A و G على التوالي. هنالك تباينا معنويا (P≤0.05) في نسبة البروتين لحليب الاغنام العواسي باختلاف التركيب الوراثي في جين FASN ولصالح النعاج ذات التركيب GG وبمعدل 5.17 %. يمكن الاستنتاج بامكانية استخدام هذا الجين في تطوير استراتيجيات للتحسين الوراثي للأغنام وتوسيع الدراسة إلى عينة أكبر ومواقع متعددة ودراسة انواع الاحماض الدهنية في الحليب وان استخراج التداخل بين التغايرين سوف يعطي نتائج أكثر دقة ويحدد أفضل طريقة لإدارة وتحسين قطعان الأغنام.

**الكلمات المفتاحية: إنتاج الحليب اليومي، طول موسم الحليب، مكونات الحليب.**



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## INTRODUCTION

A major challenge to enhance the genetic features of small flocks in emerging livestock systems is the shortage of pedigree and management, which is essential for genetic evaluation and accurate selection decisions (11). Genetic selection has grown into a significant method of genetic improvement (3, 13, 14, 19, 20). The Age of dam and the type of feed was significantly affected daily milk yield (2, 21) . The FASN gene codes for Fatty acid synthase (FASN) is a multifunctional homologous enzyme that catalyzes the production of fatty acids (FA) and is important in the synthesis of short- and medium-chain fatty acids in mammals (18, 26, 34). It also regulates the organism's energy balance and aids in the formation of milk fat during lactation (1, 7, 12, 31). The FASN gene has 42 exons and 41 introns and is 19,770 bp long (GenBank Acc. No: AF285607). The FASN is an enzyme that is engaged in tumor-related signaling pathways, the most common of which is the PI3K-AKT signaling pathway. It can also influence the immunological microenvironment, participate in the epithelial-mesenchymal transition, and govern tumor invasion and metastasis (24, 30, 33, 35). FASN is a multi-domain enzyme complex that works in the synthesis of palmitic acid via *de novo* lipogenesis and is considered a potential gene for milk production qualities in cattle (15, 17, 18, 23, 29). It has previously been revealed that FASN-driven lipogenesis failure is a common connection in insulin resistance type2 diabetes and insulin resistance metabolic disorders such as cancer, the FASN inhibitors have the potential to be used as a therapeutic agent in the treatment of obesity, cancer, and other lipid metabolic disorders(16, 28, 36). Lipid metabolism has received a lot of interest in recent years since it supplies the building blocks needed to support tumor development as well as an alternate fuel source for ATP synthesis, FASN is a major regulator of lipid metabolism that plays an important role in the development and survival of cancers with lipogenic characteristics(6, 10, 32). During lactation, FASN is essential for mammary gland growth and milk production, the mammary gland is one of the few adult organs that substantially induces *de novo* fatty acid synthesis in response to physiological stimulation, indicating that fatty acids play a major role in milk production during lactation (27). Fatty acid synthesis is the process of synthesizing fatty acids from carbohydrate and amino acid carbon sources, the majority of the enzymatic steps of fatty acid synthesis are performed by FASN (4).The purpose of this study was to identify the FASN gene polymorphism and its association with daily milk yield, lactation period, and milk composition of Awassi sheep.

## MATERIALS AND METHODS

The current study includes 52 ewes and their lambs. The ewes are fed on roughages ad libitum. The concentrated forage is also provided at an amount of 500 g/day/animal, and this quantity increases before and during the reproductive season for ewes. The samples were taken from a Sheep Farm / Al-Fayha Station in Jableh sub-district / Al-Musaib project from 5-1-2022 to 25-6-2022. (55 km south of Baghdad city). The purpose of this study was to detect the genetic information and determine the genotype of the FASN gene and its relationship to daily milk yield, lactation period, milk composition in Awassi sheep, and investigate the percentages of their genotype in the flock and the intensity of alleles obtained. Five milliliters of blood were collected from each sheep's jugular vein and placed in collection tubes with K2 EDTA anticoagulant and then carried in a cool box to the lab for cryopreservation at -4 °C. The DNA extraction from blood using the kit for extraction of DNA (Geneaid Company). The integrity of the genomic DNA was verified using agarose gel electrophoresis. The principal variant found in *FASN* gene Intron 23 is the focus of the *FASN* gene polymerase chain reaction (PCR) method. The 404 bp segment (37) identified in sheep genomic DNA was amplified using comparable primer pairs at 66.4 °C (Annealing temperature) at 32 cycles for 30 seconds, depending on the size of the fragments and the type of primers applied (forward and reverse). The primer sequences are described in full below:

F:5' CTGTTTGCTGGCACGTCCCT3'

R:5'GCACAGTGGACATCTCACCGAAGCCG3'

The detection of nucleotide sequencing for FASN gene was carried out in Nappo company using genius program. Once a month until the end of the productive season, the daily milk yield for each ewes was measured using a cylinder. In the evening, lambs were separating from their dams that are milked in the morning. Throughout a three-month period, each ewe's milk components were measured as once a month. There after, a sample was retrieved in the morning and mixed well in clean plastic containers of 50 ml capacity with tight covers that were closed after collecting the sample and sent refrigerated to the laboratory to be evaluated in the milk analyzer (Milk analyzers Julie Z7) for lactose, fat, protein, and solid non-fat, determination(19). The data was analyzed by used Statistical analysis system; SAS (25) program was used in the analysis of data to study the effect of the FASN gene polymorphism on the studied traits by applying the general linear model (GLM), according to the following statistical model. The significant differences were compared among averages by multiple range test (8).

Statistical model: (Traits on ewes).

$Y_{ijk} = \mu + G_i + A_j + e_{ijk}$

$Y_{ijk}$ = The observed value , $\mu$ = The Overall mean,$G_i$ = The effect of gene polymorphism , $A_j$ = The effect of ewe age , $e_{ijk}$= The random error.

Statistical model: (Traits on lambs).

$Y_{ijklm} = \mu + G_i + A_j + S_k + T_l + e_{ijklm}$

$Y_{ijklm}$ = The observed value , $\mu$ = The Overall mean, $G_i$ = The effect of gene polymorphism of dam , $A_j$ = The effect of age of dam at birth , $S_k$ = The effect of Sex , $T_l$ = The effect of type of birth , $e_{ijklm}$= The random error.

Chi-square ($\chi^2$) test was used to significantly compare between the percentage (0.05 and 0.01 probability) in this study. Calculator of allele frequency of FASN gene according of Hardy Weinberg's equilibrium (9).

## RESULTS AND DISCUSSION

### DNA loading and electrophoresis

DNA was separated from blood and all genomic DNA samples were loaded by Gel electrophoresis in agarose (1%), with the resultant band pattern shown in Figure 1.

**Amplification of FASN Gene by PCR:** The results showed that the amplification fragment size of FASN gene intron 23 was 404 bp (DNA ladder 100bp), All samples were amplified successfully, and a single band stained with ethidium bromide was obtained (Figure 2).

**Figure 1. Gel electrophoresis of genomic DNA extraction from blood, 1% agarose gel at 65 volts and a current of 40 mA for an hour**

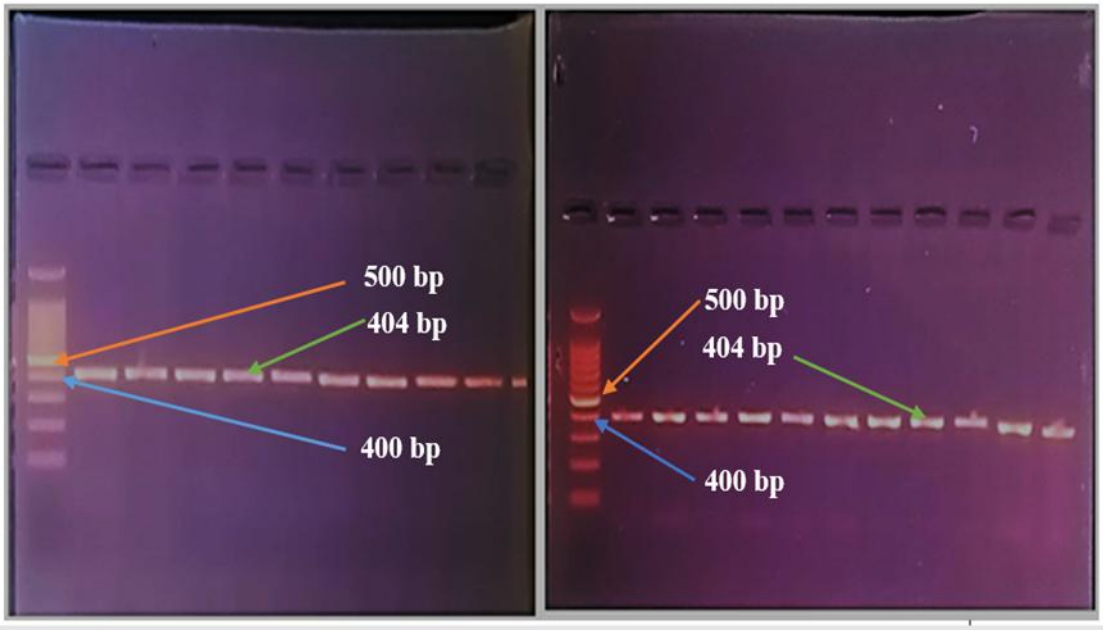


**Figure 2. PCR product band of intron 23 of FASN gene with size 404 bp agarose gels 1.5-2% ;65 V/ 60 min**

**The sequences of the nucleotides of the FASN gene:** The results revealed that the studied segment of the FASN gene (404 base pairs) had two variants (2 SNPs) in the target coding region of the FASN gene, with three genetic polymorphism appeared in the first variant (A>G SNP), which are AA, AG, and GG, and three genetic polymorphism appeared in the second variant (A>T SNP), which are AA, AT, and TT, as shown in Figure (3). Table (1) revealed the polymorphism distribution and alleles frequency of the FASN gene. Three polymorphism were found, with the percentages of AA, AG, and GG polymorphism being 63.46, 32.69, and 3.85%, and the allelic frequency of the A and G alleles being 0.70 and 0.30, respectively.

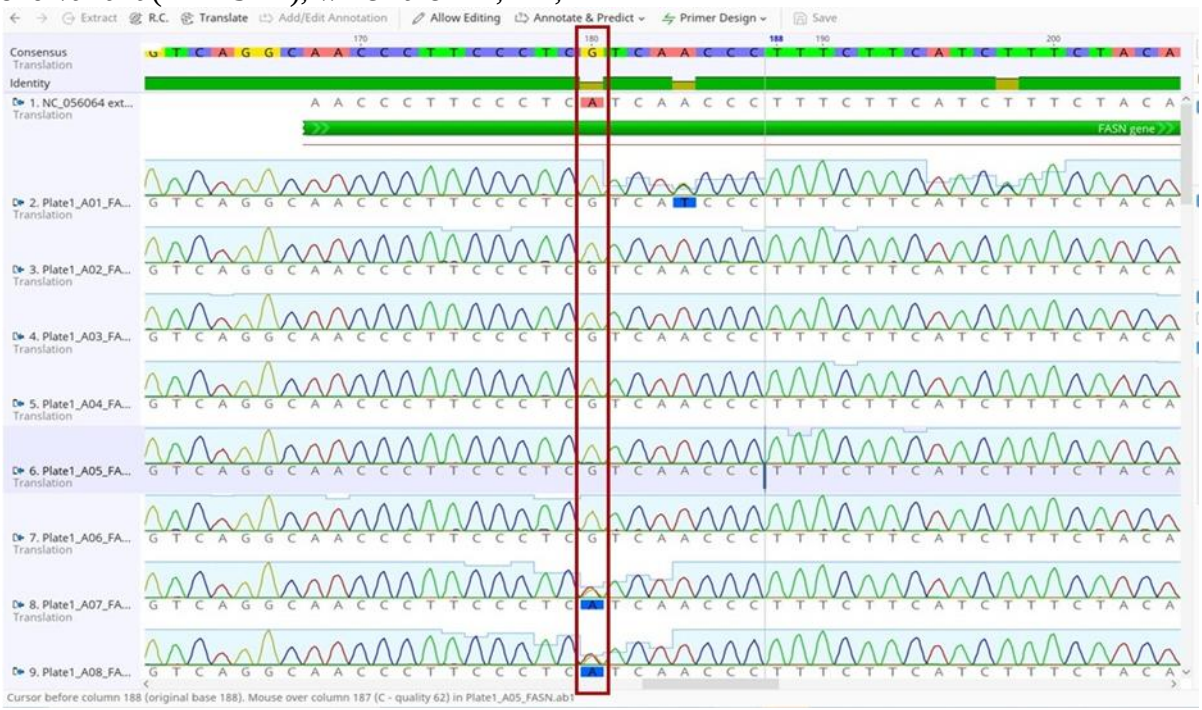


**Figure 3. Site of the variants in the FASN gene in Awassi ewes**

**Table 1. Distribution and allele frequency of FASN gene**

| FASN gene /A>G SNP Polymorphism | Number | Percentage (%) |
|---|---|---|
| AA | 33 | 63.46 |
| AG | 17 | 32.69 |
| GG | 2 | 3.85 |
| Total | 52 | 100% |
| Chi-Square ($\chi^2$) | - | 43.192 ** |
| Allele | Frequency | |
| A | 0.70 | --- |
| G | 0.30 | --- |

** (P≤0.01).

**/A>G SNP Polymorphism in Awassi ewes**

**Relationship of FASN gene with daily milk yield-DMY and lactation period of SNP (A>G) polymorphism**

The results showed that non significant differences among the AA, AG, and GG polymorphism, being 1022.22 ± 17.38, 981.37 ± 24.88 and 1004.17 ± 4.17 (gr), respectively, for DMY (gr), as well as 120.77 ± 11.42, 108.36 ± 7.69, and 119.53 ± 9.57. (day) respectively for lactation period (Table 2).

**Table 2. Relationship of FASN gene /A>G SNP1 polymorphism with daily milk yield-DMY and lactation period**

| FASN gene /A>G SNP1 Polymorphism | Mean ± SE | |
|---|---|---|
| | DMY (gr) | Lactation period (day) |
| AA | 1022.22 ±17.38 | 120.77 ±11.42 |
| AG | 981.37 ±24.88 | 108.36 ±7.69 |
| GG | 1004.17 ±4.17 | 119.53 ±9.57 |
| Level of Sig. | NS | NS |

NS: Non-Significant.

Jawasreh and Khasawneh (13) explain that, the milk yield of Awassi ewes is affected by the dam's age and weight at lambing, the duration of the lactation period., the type of birth, the sheep management practices, the month of lambing, and the sex of the born lamb, they indicated that the overall mean of daily milk yield 0.796 ± 0.0086 kg. Capistrak et al. (5) shown that environmental conditions had a significant impact on variance in season duration and daily milk yield, primarily among individuals of the same breed.

**Relationship of FASN gene and milk composition of SNP1 (A>G) polymorphism**

The results revealed that there were significant differences (P<0.05) between the different polymorphism in the percentage of milk protein, as the GG polymorphism (5.17 ± 0.26) was significantly superior for the GG and AG polymorphism (5.01 ± 0.09, 4.58 ± 0.17), respectively, while milk fat, milk lactose, and solid nonfat, did not exhibited any significant differences among the different polymorphism (Table 3).

**Table 3. Relationship of FASN gene /A>G SNP1 polymorphism and milk composition (Mean ± SE)**

| FASN gene /A>G SNP1 Polymorphism | Milk composition | | | |
|---|---|---|---|---|
| | fat (%) | lactose (%) | protein (%) | Solid nonfat (%) |
| AA | 5.41 ±0.31 | 5.02 ±0.07 | 5.01 ±0.09 ab | 10.90 ±0.13 |
| AG | 5.84 ±0.42 | 5.07 ±0.10 | 4.58 ±0.17 b | 10.36 ±0.19 |
| GG | 5.93 ±0.95 | 4.90 ±0.14 | 5.17 ±0.26 a | 10.72 ±0.02 |
| Level of Significant | NS | NS | * | NS |

Means having with the different letters in same column differed significantly. * (P≤0.05), NS: Non-Significant.

The significant effect of the AA genotype on the percentage of milk protein is of great importance in the genetic selection process to produce individuals carrying this type that are characterized by an increase in the percentage of protein in their milk, as milk protein is of great importance in providing the human body with essential amino acids. Suburu et al. (27) reported that FASN removal significantly decreased the medium- and long-chain fatty acid and total fatty acid content in milk, The development, function, and maintenance of the

lactating mammary gland depend on FASN. According to Pecka-Kiełb et al. (22), the *FASN* gene function is an essential in fatty acid metabolism and/or its presence in cytogenetic areas related with milk quantity and quality, SNP variations within this gene can explain some of the variance in Fatty acid component in sheep milk. The present investigations on the association of intended coding regions in the *FASN* gene that the *FASN* gene has a specific function in several of the analyzed trait, particularly the percentage of milk protein.

**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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