Eksplorium *p*-ISSN 0854-1418
Volume 46 No. 1, May 2025: 747–755 *e*-ISSN 2503-426X

Impact of FASN Gene Genotypes on Some Milk Components and Udder Dimensions Traits in AWASSI Sheep

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Article Received: 26 Feb 2025, Revised: 22 April 2025, Accepted: 05 May 2025

Abstract: This experiment was conducted at the Sheep and Goat Research Station located in Al-Shatra District, north of Thi Qar Governorate, affiliated with the Thi Qar Agriculture Directorate. The study lasted from October 11, 2024, to March 26, 2025, and included 40 Awassi ewes.It aimed to study the effect of genotypes of FASN gene on milk components traits, in addition to the udder dimensions characteristics, using Sequencing technology.The field aspect included collecting milk samples and measuring udder dimensions (udder length, udder width, udder circumference, teat length, teat circumference). As for the laboratory part, the proportions of milk components were measured, including (fat, protein, lactose, non-fat solids, density, freezing point, and temperature), and DNA was also analyzed to detect changes in the FASN gene. Sequencing results in the region of exons 37,38 and 39 with a length of 617 base pairs showed no mutations, while the region of exons 18,19 and 20 with a length of 997 base pairs revealed a C>G mutation at position 562 within exon 20, Causing the amino acid to change from Leucine to Valine, this is a type of mutation known as a missense mutation. Three genotypes (CC, CG, GG) were identified with significant differences in their distribution, where the wild type (CC) was the most common (53.1%), and the frequency of the C allele was higher (0.69) than the G allele (0.31). The results showed significant differences in the percentage of milk fats exceeding the GG composition and the density of milk exceeding the CG composition, while no differences were observed in the rest of the components. GG also outperformed udder girth and CG in udder length, with no differences in other traits. It can be concluded from this study that the FASN gene can be used as a genetic marker to improve productive performance in Awassi sheep by selecting genotypes that achieve the best productive performance in milk and its components.

Keywords :FASN gene, milk components, udder dimensions, Awassi sheep

INTRODUCTION

Awassi sheep constitute 58.2% of the total sheep population in Iraq, and are raised for meat, wool, and milk production. They are characterized by their tolerance to harsh environmental conditions, with variations in their productive and reproductive characteristics depending on the environment and region in which they are found (Salman and Abdalla, 2014). The dairy sheep industry is an essential yet underutilized segment of the small ruminant sector. Sheep milk can be utilized to produce high-quality dairy products, contributing to local or international economic benefits (Li et al., 2022). Morphological characteristics of the udder, such as udder and teat dimensions, are important factors associated with milk production performance and its components in Iraqi Awassi sheep, as these measurements contribute to the accurate prediction of production performance (Al-Hubaiti, 2009). To achieve continuous improvement in Awassi sheep breeds, updating genetic improvement methods through studying the genotypesand analyzing genes associated with growth and production traits is essential, The study of mutations and their phenotypic association using advanced techniques such as PCR, RFLP, and DNA sequencing. These

procedures aim to identify genes that influence and enhance economic traits (Shokrollahi, 2015; Salim and Abdulkareem, 2019; Thbit, et all.,2021).Fatty acid synthase (FASN) is a multifunctional isoenzyme, encoded by the FASN gene, that plays a vital role in the synthesis of short- and medium-chain fatty acids in mammals (Crisà et al., 2010; Urrutia et al., 2020). FASN is considered an acid-synthesizing enzyme essential for mammary gland growth and milk production, which confirms the importance of these acids in supporting the vital process of lactation (Subur et al., 2014). The aim of our study was to demonstrate the effect of FASN gene genotypes on a number of milk component traits and udder dimensions in Awassi sheep.

MATERIALS AND METHODS

The study was conducted at the Sheep and Goat Research Station located in Al-Shatra District, north of Thi Qar Governorate, affiliated with the Thi Qar Agriculture Directorate within the Ministry of Agriculture. The study continued during the period from 10/11/2024 to 3/26/2025, and included 40 ewes of the Awassi sheep breed. Data on the sheep used in the experiment (animal ages, weights, and other station records) are collected. The study included measurements of udder dimensions (udder length, udder width, udder circumference, teat length, teat circumference). Milk samples were collected from each animal during the morning milking, where the samples were placed in dedicated tubes after mixing the milk produced from each ewe well to ensure sample homogeneity, in an amount of approximately 50 ml. Milk components, including fat, protein, lactose, solids-non-fat, density, freezing point, and temperature, were measured using a LACTOSCAN analyzer from the 40th day after lambing until the end of the production season. Then, blood samples were taken to detect the genotypes of the FASN gene, 3 ml of blood was collected from the jugular vein of each animal using a sterile syringe and placed in a collection tube containing EDTA anticoagulant.

The animal number was recorded in each individual tube and frozen. For the extraction process (laboratory part) to separate the genetic material in the laboratory of the Marshlands Research Center / University of Thi Qar. DNA extraction was performed using Geneaid Kit to conduct molecular testing of FASN gene, and the resulting RNA was electrophoresed at 70 volts and 85 mA for 20 minutes using electrophoresis technique. Then, the agarose gel was examined after the migration time was over using a UV Gel Documentation device, and migration images were taken using the installed camera. After completing the electrophoresis process of the PCR product, a UV Gel Documentation device was used to image the products, in order to verify the success of the DAN extraction process, and obtain the targeted gene fragments. FASN gene-specific primers were prepared by Macrogene, a Korean company, for molecular screening and identification of phenotypic polymorphisms and mutations in the FASN gene.

Table No. (1) shows the primers used in the experiment.

Gene	primers	Piece size	Reference
	F: GACAGCTCGCTTTCAGACCT		This study

FASN Gene	R: AGGCCCCTGACATACCTCTT	617 base pair	
	F: CCTGCACCTTTGAGGTGTCT	997 base pair	This study
	R: CCGGCATGAGGATTTTGGGT		

Agarose gel was prepared using the same steps as the previous DNA removal steps for the samples, but the concentration of agarose prepared for the removal of PCR product samples was 1.5%.

Table (2) PCR program for the FASN gene.

Gene	Stages	Temperatures	Time (minutes)	Number of cycles
FASN	Initial denaturation	95C°	5	1
	denaturation	95C°	0.30	35
	annealing	55 C°	0.30	
	Elongation	72C°	0.45	
	Final Elongation	72C°	10	1

After confirming the size of the PCR product specific for the FASN gene by comparing it with the standard DNA strip (DNA ladder), 20 microliters of each sample were taken from the PCR product and sent to the Korean company Macro gene, The samples were purified and then sequenced using Sanger sequencing. The sequence results were received and analyzed using BLAST tools on the NCBI Gene Bank website, along with some bioinformatics programs. The study data were statistically analyzed using the ready-made statistical program SAS (Statistical analysis system) (SAS, 2018), in which the potential effect of the genotypesof the FASN gene on the traits under study was studied, according to the mathematical model below, the significant differences between the means were compared using the Duncan multiple range test (Duncan, 1955) and using the completely randomized design (CRD). The percentages of the distribution of the genotypes of the gene were compared using Chi-square and calculating the allelic frequency as in the following equation:

PA=2×NO.of homozygous + no. of heterozygous /2 × total of samples
P+q=1 , then : qB= 1-Pa
$\chi^2 = \Sigma [(O - E)^2 / E]$

RESULTS AND DISCUSSION

After verifying the success of the DNA extraction process, the studied gene fragments of the FASN gene were amplified and multiplied using PCR technology, Use the PCR kit and primers for kidney DNA samples and set up the thermal cycler, The PCR product was then

electrophoresed in a 1.5% agarose gel for the FASN gene. The migration program was set using 70 volts and 85 mA for 45 minutes. After completing the electrophoresis of the PCR product, a UV Gel (Documentation) device was used to image the products, in order to verify the success of the amplification process, and obtain the target fragments.

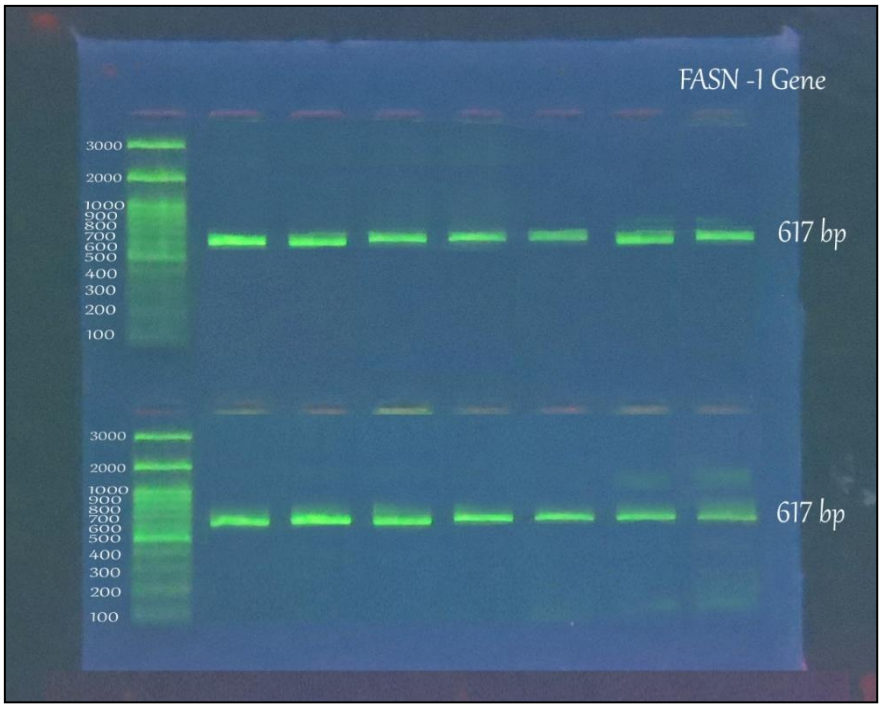
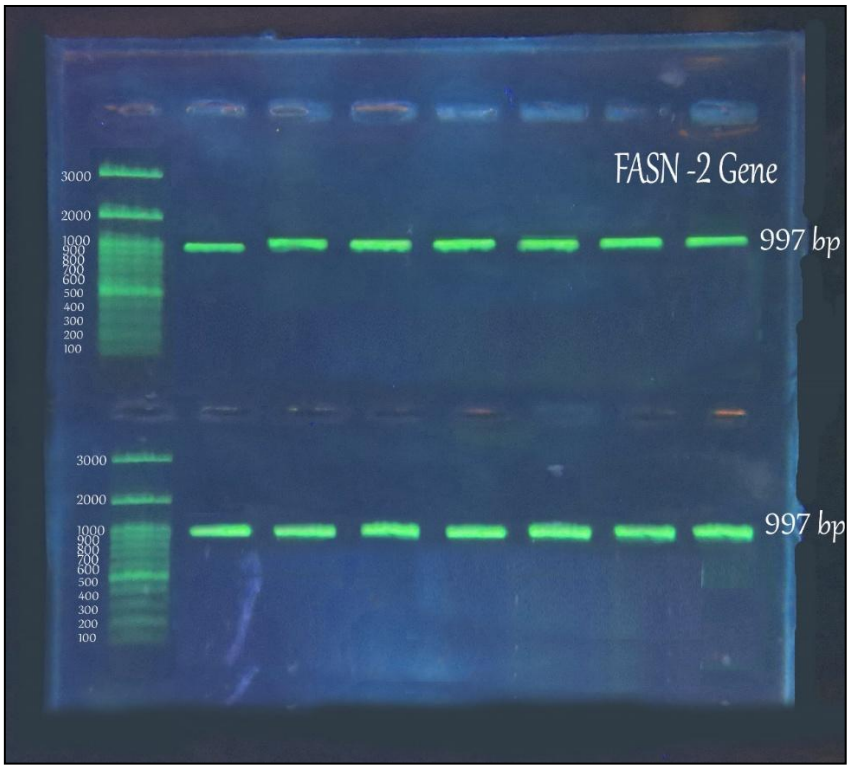


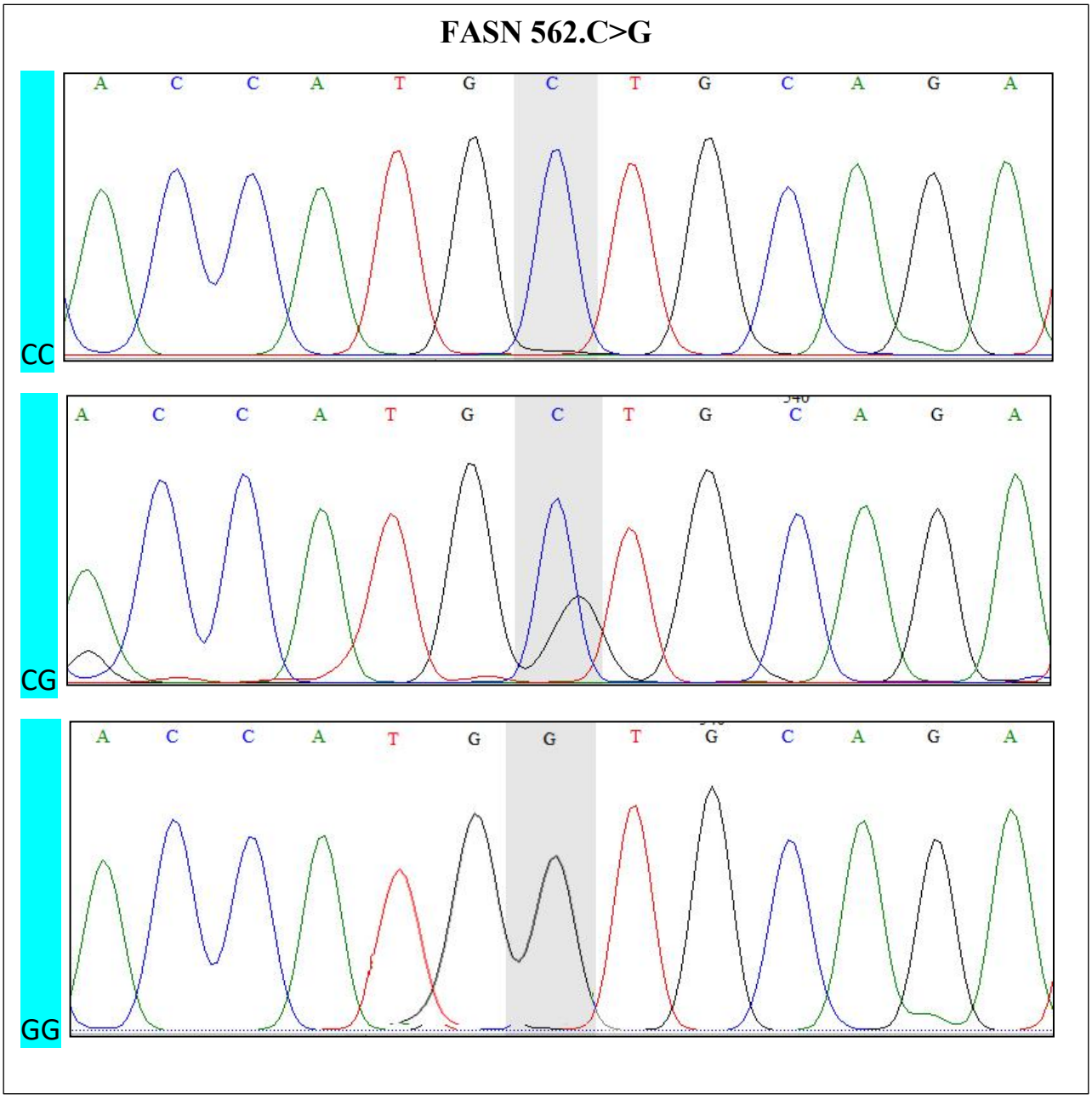
Image (1) Electrophoresis of the studied region (1) of the FASN gene with a size of (617 base pairs) and the stage on a 1.5% agarose gel.



p-ISSN 0854-1418
e-ISSN 2503-426X

Image (2) Electrophoresis of the studied region (2) of the FASN gene, with a size of (997 base pairs) and the stage on a 1.5% agarose gel.

The results of the sequencing revealed that the targeted gene fragment, which was 617 base pairs in size, included exons 37-38-39. All genotypes in this segment were found to be identical, and no change in the nitrogenous base sequence was observed in the Awassi sheep sample. The second region in the 997 base pair gene segment included exons 18-19-20, and the results of its study revealed the presence of a single mutation (C>G at position 562) within the studied region of the FASN gene. The mutation occurred in exon 20, which consists of 59 amino acids. The code for the amino acid (Leucine) CTG was changed to GTG, and the amino acid was changed to (Valine), which is located at position 19 of the peptide chain of the FASN protein in exon 20, This type of mutation is known as a missense mutation, because it results in a change in the amino acid. This change may have an effect on the structural or functional composition of the FASN protein, as shown in Figure (1).



It is noted from Table (3) that there are highly significant differences (P≤0.01) in the distribution ratios of the different genotypes of the FASN gene (mutation (562.C>G) in Awassi ewes, The percentage of these combinations reached 53.8, 30.8 and 15.4% for the CC, CG and GG genotypes, respectively, It is clear that the percentage of animals with the wild genotypes CC was the highest among the hybrid genetic makeups CG and the mutant GG. Table 3 shows the presence of three genotypes, namely CC, CG, and GG, linked to the C and G alleles of the FASN gene in a sample of Awassi sheep. The frequency of the C allele was 0.69, while the frequency of the G allele was 0.31, indicating a clear prevalence of the C allele in this sample.

Table (3): Frequency of genotypes, number, percentage, and frequency of alleles in the mutation C>G.562 of the FASN gene in Awassi sheep.

Genetic makeup	Number	percentage (%)	chi-square value
CC	21	53.8	
CG	12	30.8	
GG	6	15.4	
Total	39	100%	17.3077**
Gene alleles			
C	0.69		
G	0.31		
Probability level	** P≤0.01		

Tables (4 and (5) show the relationship between the genotypesof the FASN gene and the characteristics of milk components in Awassi sheep, Table 1(4) shows that there are significant differences (P≤0.05) in the percentage of fat in milk between the three genetic compositions, as the mutant genetic composition GG outperformed the wild (5.734%) and hybrid (4.166%) compositions by an average of 6.387%, This result did not agree with what was stated by Naima and Al-Anbari (2022) in terms of the appearance of insignificant results in the percentage of fat in Awassi sheep. The current study also contradicted what Al-Maamory and Al-Anbari (2023) reached, as they concluded that there were no significant differences between the studied genetic compositions in the milk fat trait in Awassi sheep, From the same table, it was noted that there were no significant differences between the three genetic compositions in the milk protein trait, despite the simple arithmetic superiority of the mutant genetic composition GG (5.032%) over the hybrid and wild genetic compositions, This result is consistent with what Al-Maamouri Al-Anbari (2023) reached. Contrary to what Naima and Al-Anbari (2022) found, there were significant differences in the various genetic compositions of the milk protein percentage trait in Awassi sheep.

It is also clear from Table 1 (4) for the proportions of other components represented by the percentage of sugar and non-fat solids that there are no significant differences according to the genotypes of the FASN gene, and these results are consistent with the findings of (Thomas et al. (2001); Clément et al. (2006); Naima and Al-Anbari (2022); and Al-Maamory and Al-Anbari (2023)).

Table 2 (5) shows the presence of highly significant differences (P≤0.01) in the percentage of milk density between the genetic compositions of the FASN gene, as the hybrid genetic composition CG is superior And at a rate of 43.607% on the two genotypes, the mutant GG (39.480%), and the wild CC (34.815%), While no significant differences were found in the characteristics of the other components (milk freezing point and milk temperature). Sabahelkhier et al. (2012) determined the protein content of sheep milk as 6.35%, fat 6.90%, sugar 5.00%, and dry matter 19.03%, The percentage of fat in our current study was within this range, while the percentages of the remaining milk components, protein, sugar and solid matter, were significantly lower compared to these values, This difference may be related to multiple factors such as animal maintenance and feeding methods, lactation stage, age, and health status (Tomas et al., 2001; Kuchtik et al., 2008; Pecka et al., 2013).

Table (4): The relationship of the genotypes of the mutation 562. C>G in the FASN gene with the characteristics of milk components (1) (mean ± standard error).

Genotype	Number	Mean ± Standard Error			
		Fat percentage	Protein percentage	sugar percentage	Non-fat solids
CC	14	5.734 ± 0.413 ab	4.511 ± 0.255	4.520 ± 0.159	9.943 ± 0.432
CG	8	4.166 ± 0.315 b	4.877 ± 0.190	4.750 ± 0.134	10.692 ± 0.291
GG	4	6.387 ± 1.463 a	5.032 ± 0.343	4.777 ± 0.327	10.577 ± 0.698
Significance level	26	*	NS	NS	NS

No significant N.S (P≤0.05) *

Table (5): The relationship of the genotypes of the mutation 562. C>G in the FASN gene with the milk component traits (2) (mean ± standard error)

Genotype	Number	Mean ± Standard Error		
		Milk density	Freezing point of milk	milk temperature (C^0)
CC	14	34.815 ± 1.961 b	0.575 ± 0.026	22.442 ± 0.471
CG	8	43.607 ± ± 0.368 a	0.584 ± 0.020	24.650 ± 1.050
GG	4	39.480 ± 4.323 ab	0.558 ± 0.033	22.100 ± 0.807
Significance	26	**	NS	NS

level				

No significant N.S (P≤0.05) *

Effect of the mutation C>G 562 of the FASN gene on udder dimension traits in Awassi sheep. The results of Table (6) showed a highly significant effect (≤0.01 P) of the different genetic compositions CC, CG, GG of the FASN gene in Awassi sheep, on the udder circumference trait, where the ewes with the mutant genetic composition GG 41.698 cm were superior On the hybrid CG 39.370 cm and the wild CC 33.927 cm. It was also noted that the udder length trait had a highly significant effect (P≤0.01) and superiority of the hybrid genotype CG (20.743 cm) over the mutant genotypes GG (20.531 cm) and the wild genotype CC (15.602 cm). There were no significant differences between the CC, CG and GG genotypes in the traits of udder width, teat length and teat diameter, despite the observation of a high average for the mutant GG genetic structure (although not significant) in these traits. This indicates the possibility of this structure being superior if the sample size was larger than the current sample size.

Table (6): Relationship of genotypes of the mutation C>G 562 in the FASN gene with udder dimension traits (cm) (mean ± standard error)

genotype	Number	Mean ± Standard Error				
		Udder circumference	Udder length	Udder display	Nipple length	Nipple diameter
CC	21	33.927± 1.112 b	15.602± 1.221 b	11.057± 0.173	4.233 ± 0.291	4.505 ± 0.401
CG	12	39.370± 2.308 ab	20.743 ± 1.282 a	11.633± 0.148	4.021 ± 0.361	4.497 ± 0.370
GG	6	41.698± 1.808 a	20.531± 2.084 a	12.700 ± 0.288	4.656 ± 0.453	5.080 ± 0.714
Significance level	39	**	**	NS	NS	NS

No significant N.S (P≤0.05) *

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