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Genetic polymorphism of FASN gene and its relationship to some body dimensions, growth, and fertility traits in Awassi sheep

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Abstract

The study was conducted at the Sheep and Goat Research Station in Al-Shatra District, north of Thi-Qar, affiliated with the Thi-Qar Agriculture Directorate, and included 39 ewes of the Awassi sheep breed. The study aimed to analyze the genotyps of the FASN gene using the sequencing technique and to determine its relationship with growth traits, body dimensions, and fertility rate. DNA sequencing results in exons (39-38-37, 617 base pairs long) showed no mutations, while sequencing of exons (20-19-18) of 997 base pairs revealed a C>G mutation at position 562 within exon 20, resulting in an amino acid change from leucine to Valine. It is a missense mutation. Three genotypes have been identified (CC, CG, GG). The wild type (CC) was the most common (53.1%), with an allelic frequency of 0.69 for the C allele and 0.31 for the G allele. The results showed a significant effect of FASN gene genotypes on the rear height trait; CG ewes outperformed GG and CC ewes. A significant effect was also recorded on the birth weight of the fetus, with the GG combination being superior, without any significant differences being recorded in the fertility rate between the three combinations. The study concludes the importance of the FASN gene as a genetic indicator for improving productive performance in Awassi sheep by selecting genetic combinations associated with the best productive traits.

Keywords :FASN gene, Body dimensions, Fertility, Awassi sheep

I. Introduction:

The Awassi sheep breed is one of the most widespread sheep breeds in the Middle East, due to its high ability to adapt to harsh environmental conditions (AI_Thuwaini 2021).In addition, this breed is characterized by high meat and milk productivity, which distinguishes it from other breeds prevalent in the region (Ajafar et al., 2022). Growth performance is considered a key factor in enhancing the profitability of the livestock production industry, as rapid growth contributes to increasing animal weight, thus enhancing meat production and economic returns, which requires its careful evaluation to achieve production efficiency (Singh et al., 2016 and Thbit et al., 2021).

Measuring animal body dimensions can be used to predict some production traits, such as live body weight during fattening periods. It also helps in determining the appropriate periods for the fattening process (Al-Mahdawi., 2011; Salim and Al-Zaydi., 2021).Genes are the basic units of a living organism's body, playing a pivotal role in regulating vital functions and chemical reactions, and directing the stages of growth and development. It also contributes to determining qualitative and quantitative traits through interaction with the environment, and mutations in it may lead to various diseases (Deacon., 2022 and Bicknell et al., 2012).FASN is a large, 270 kDa, multi-enzyme complex with six enzyme sites that work together to produce palmitic acid, a saturated fatty acid, using acetyl-CoA and malonyl-CoA to build a 16-carbon chain (Ventura et al., 2015).

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The FASN gene is considered a promising candidate for regulating the lipid composition of cattle meat, due to its important role in controlling fatty acid composition, which contributes to improving meat quality and nutritional properties (Kaplanová et al., 2013; Al-Thuwaini et al., 2022).In sheep, it consists of 42 exons and is located on chromosome 11, where it regulates the Thioesterase TE domain within the FASN complex to terminate fatty acid synthesis (Esteves et al., 2019). The objectives of the research were to study the effect of genetic conformation of the FASN gene on body dimensions, growth, and fertility traits in Awassi sheep.

II. Materials and Methods

This experiment was conducted at the Sheep and Goat Research Station located in Thi Qar Governorate, Shatrah District, affiliated with the Thi Qar Agriculture Directorate. It included 39 ewes of the Awassi sheep breed. The study continued during the period from 11/10/2024 to 26/3/2025. Data on sheep used in the experiment were collected from station records. The herd is managed according to an integrated program that includes feeding, the breeding season, preparation for pregnancy and birth, and the provision of health and veterinary care.

The experiment included two main parts: The first included the ring measurements of the studied traits. The body dimensions of the ewes were measured (body length, height at the front, height at the rear, chest width, chest circumference, and belly circumference), Then, 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 tube and stored in a freezer. For the extraction process (laboratory part) to separate the genetic material in the laboratory of the Marshlands Research Center / University of Dhi Qar. While the second part is on the laboratory side, where blood samples were drawn to detect the genetic makeup of the FASN gene. DNA was extracted (Geneaid Kit) for molecular testing of the FASN gene, Then, I relayed the output using 70 volts for 85 milliamps for 20 minutes using the electrophoresis technique, The agarose gel was examined after the migration time using a UV Gel Documentation device and migration images were taken using the mounted 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 pieces of the gene. FASN gene-specific primers were prepared by Macro Gene, 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	Source
FASN Gene	GACAGCTCGCTTTCAGACCT	617 base pair	This study
	AGGCCCCTGACATACCTCTT		
	CCTGCACCTTTGAGGTGTCT	997 base pair	This study
	CCGGCATGAGGATTTTGGGT		

Agarose gel was prepared using the same steps used to remove the DNA from the samples, but the concentration of agarose prepared for the removal of PCR product samples was 1.5%.

Table No. (2) PCR program for the FASN gene.

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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 to the FASN gene by comparing it with the standard DNA ladder strip, 20 microliters of each sample were taken from the PCR product and sent to the Korean company Macrogene, where the samples were purified and then analyzed using sanger sequencing technology,The base sequence results were received and analyzed using BLAST tools at the NCBI GenBank website along with some bioinformatics software.

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 genetic makeup of the FASN gene on the traits under study was studied according to the mathematical model below. Significant differences between 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 the 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

$\chi^2 = \Sigma [(O - E)^2 / E]$

III. 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, using a PCR kit, primers, and total DNA samples, and adjusting the thermal cycler. The PCR product was then electrophoresed in a 1.5% agarose gel for the FASN gene. The electrophoresis program was set using 70 volts and 85 mA for 45 minutes. 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 amplification process and to obtain the target fragments.

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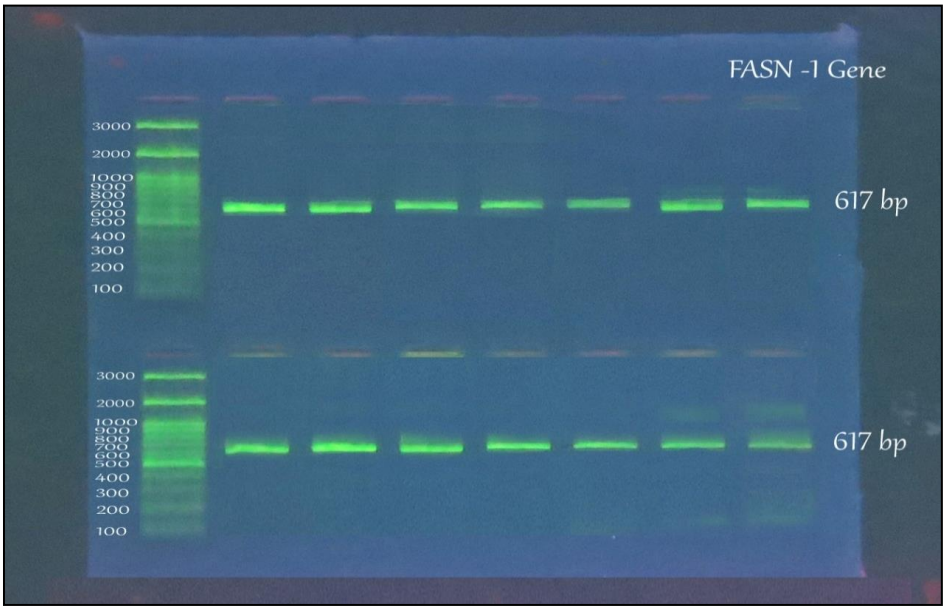

Fig. (1) Electrophoresis of the FASN gene with a size of (617 base pairs) and the stage on a 1.5% agarose gel.

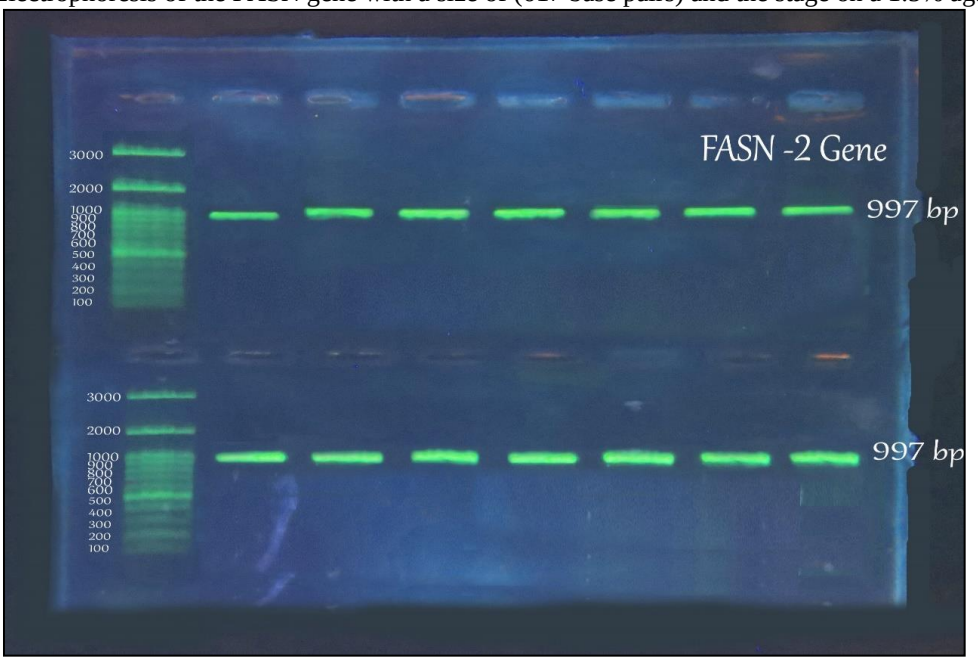

Image (2) Electrophoresis of the FASN gene with a size of (997 base pairs) and the stage on a 1.5% agarose gel.

The results of the sequencing revealed that the targeted gene fragment, which was 617 base pairs in size, included exons 39-38-37. 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)

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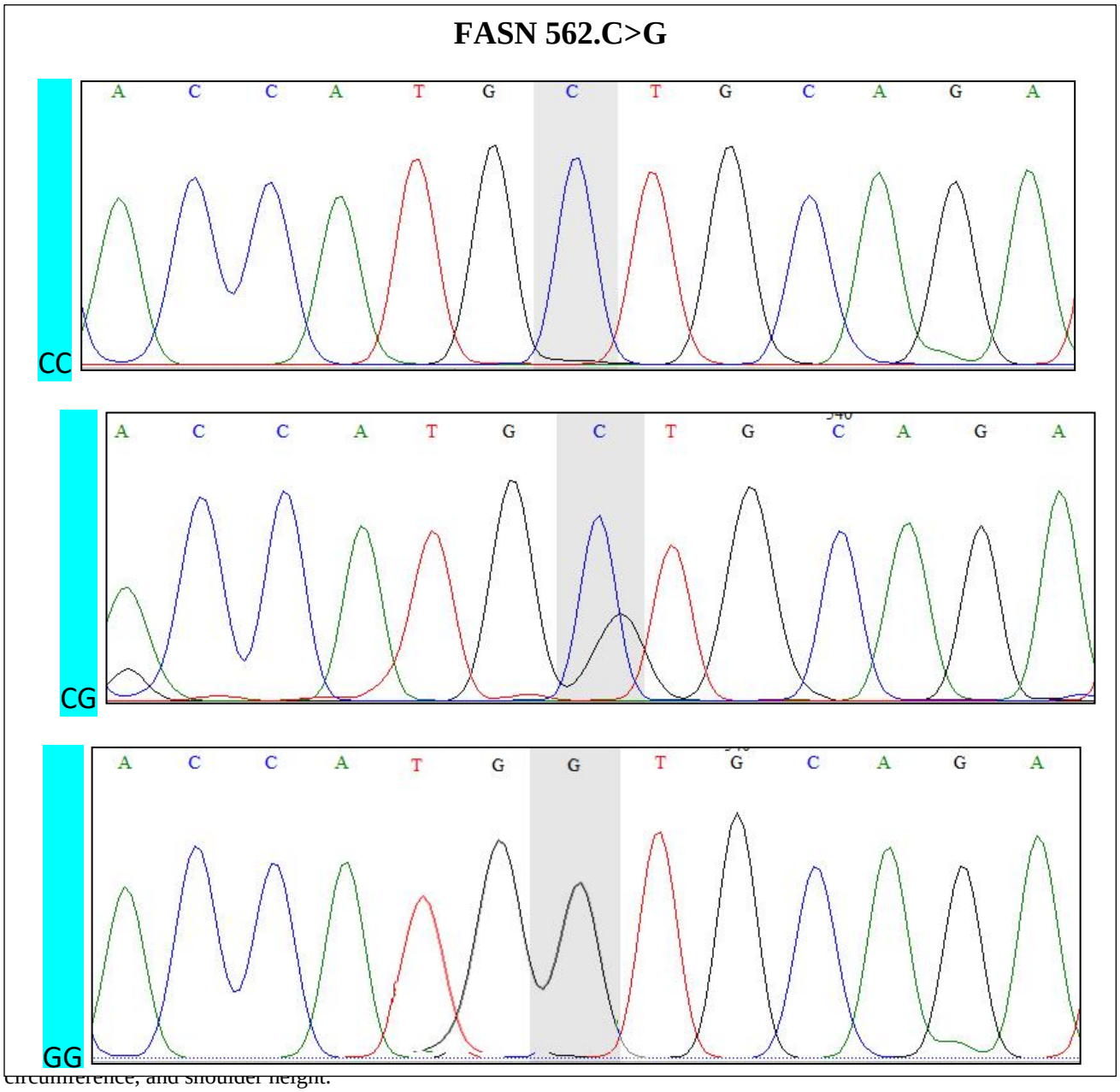
within the studied region of the FASN gene. The mutation occurred in exon 20, which consists of 59 amino acids. The code for the amino acid (leucine) CTG was changed to GTG, and the amino acid was changed to (valine), which is located at position 19 of the peptide chain of the FASN protein in exon 20, 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).



circumference, and shoulder height.

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Table (3): Relationship of genotypsof the C>G mutation in the FASN gene with body dimensions (cm) (mean ± standard error)

genotype	number	Mean ± Standard Error					
		Body length	Front hight	rear hight	Chest width	Chest circumference	Abdominal circumference
CC	21	65.132± 1.041	73.418± 0.796	68.761 ± 0.596 ab	18.505± 0.500	83.094 ± 1.096	99.422 ± 1.985
CG	12	64.346± 1.097	75.247± 1.050	70.379 ± 0.740 a	18.838± 0.660	86.360 ± 0.988	103.716± 1.204
GG	6	67.733± 0.535	71.966 ± 0.846	67.733± 1.120 b	19.261 ± 0.509	83.820± 1.854	104.986± 2.240
Morale level	39	NS	NS	*	NS	NS	NS

No significant N.S (P≤0.05) *

Effect of the C>G.562 mutation of the FASN gene on birth weight and fertility

Table (4) shows significant differences (P≤0.05) in the weight of the lamb at birth (as a maternal trait) according to the genetic compositions of the FASN gene, as the mothers with the mutant genetic composition GG (3.15 kg) outperformed the hybrid genetic compositions CG (2.83 kg) and the wild CC (2.88 kg). Naima and Al-Anbari (2022) indicated that the weight of lambs at birth and at weaning, in addition to the rate of weight gain between them, are among the basic vital characteristics that are taken into consideration when seeking to improve growth characteristics in sheep flocks, The results of his study strongly support the possibility of adopting genetic analysis of this FASN gene within selection programs, given the presence of a positive genetic correlation between body weights in the early stages and body weights and measurements in later stages of growth. This contributes to accelerating genetic improvement and increasing economic returns. There is a close association between the FASN gene and growth traits, especially weight, which is a key trait used as a criterion for selecting offspring (Boligon et al., 2011).The results from Table 4 also indicated the absence of significant differences in the fertility rate trait according to the three genotypsof the FASN gene. This result was consistent with what was reached by Kawaguchi et al. (2020) and Gonzalez Berrios et al. (2024) in their study on cows, who indicated that there were no significant differences in the fertility trait for the same gene studied.

Table (4): Relationship of the genotypes of the mutation 562. C>G in the FASN gene with the characteristics of lamb weight and fertility rate (mean ± standard error)

genotype	number	Mean ± Standard Error	
		lamb weight (kg)	Fertility rate (%)
CC	21	2.880 ± 0.050 b	66.6 ± 0.105
CG	12	2.833 ± 0.094 b	66.6 ± 0.142
GG	6	3.1583 ± 0.082 a	66.6 ± 0.210
Morale level	26	*	NS

No significant N.S (P≤0.05) *

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