FASN gene polymorphism and its relationship with milk yield and Composition in the Iraqi Awassi sheep

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Abstract

This study was conducted at the Barakat Abu Fadl al-Abbas (peace be upon him) sheep breeding station / Al-Kafeel General Investment Company in Karbala Governorate for the period from 1/12/2020 to 1/6/2021, with the aim of determining the Frequency of Alleles and Genetic Manifestations of the FASN gene and its relationship to milk production and its main components from The percentage of fat, protein, lactose and non-fat solids in Iraqi Awassi sheep, 51 female Awassi sheep were used in this study. The percentage of distribution of genotype for the FASN gene in the studied Awassi sheep sample was 7.84, 21.57 and 7.59% for each of the structures AA, AC and CC, respectively, and the two alleles were A and C, with a frequency of 0.19 and 0.81. respectively, significant differences were found in total milk production. As for the milk components, only the protein percentage was affected significantly by the different genotype, and there was no significant effect for the other components by the different genotype of the FASN gene. From the results of the current study, it can be concluded that the FASN gene can be adopted as an important indicator in the development of genetic improvement strategies for Awassi sheep to maximize the economic return from breeding projects by selecting and cross-breeding the genotypes that achieved the best productive performance.

Keywords: FASN gene, Genotype, Milk production and components.

Introduction

Reliance on Molecular Markers to characterize genetic resources is important because of the stability of their results, their independence from environmental changes and the possibility of benefiting from them in analyzes of genetic diversity, and the development in molecular biology techniques gave way to the possibilities of selection and genetic improvement of farm animals [1]. Genes are one of the basic structures of the organism's body, and through them the body passes through multiple stages, as it enhances the ability of the organism in different stages, as it is responsible for the vital functions in the body, and genes enable the organism to follow the internal chemical

reactions in the body, and the functions of different Genes from one place to another in the body, they are largely responsible for delivering the body to important stages of its life, because genetic mutations may cause several diseases, and the gene is what determines everything in the organism, starting from the formation of qualitative and quantitative characteristics (overlapping with the environment) and ending with general health [2]. Significant progress has been made in sheep breeding and improvement in the past few decades, but achieving a better understanding in improving the quality of production performance was too slow before genetics and molecular markers became an accessible technology with wide applications in breeding methods [3]. The identification of genetic markers related to meat and milk production is the main objective of studies conducted on the sites of quantitative traits. The Fatty acid synthase (FASN) gene in sheep has been identified on chromosome 11 and consists of 42 exons [4], a multienzyme protein responsible for the synthesis of acids. Fatty acids in milk, as it has been proven that it has a role in stimulating the synthesis of fatty acids in mammals, so it is considered a functional candidate gene for various because it affects the metabolism processes because it affects the composition of fat in milk, as it stimulates the last step of biosynthesis of fatty acids [5], and depends on milk production And its components on the nutrients available in the blood, which are closely related to each other, and in this regard comes the traits with a high genetic equivalent, such as the percentage of fat and protein. The genetic morphology of this gene was considered evidence of the relative differences between individuals for the above traits [6]. In view of the scarcity of studies on the relationship of polymorphism of the FASN gene in some economic traits in the local Awassi sheep, the current study aimed to determine the polymorphism of the FASN gene and its molecular role in the sample of the studied Iraqi Awassi sheep and to relationship of polymorphism of the FASN gene and growth traits in order to assess the possibility of benefiting from them as indicators for the election.

Materials and Methods

The study included a biochemical part and a genetic part, and the biochemical aspect included measuring the chemical composition of milk and was conducted at the Al-Fadhel Foundation for Study Services, Training and Development. As for the genetic aspect, genetic laboratory analyzes were conducted in the laboratories of scientific progress specialized in molecular genetics and biotechnologies, with the aim of separating the genetic material and determining the Genotypes of the FASN gene.

Study Animals

used In this study, 51 female Awassi sheep were used, aged between 8-4 years, and the system followed in raising sheep is in semi-open pens (40% roofed and 60% open) designated to house them, and the herd is managed according to a program that includes feeding and preparation for the dowry season.Preparation for pregnancy and childbirth, as well as health and veterinary care.

Blood samples

3 ml of blood was collected from the jugular vein of each animal in a collection tube containing EDTA K3 inhibitor. ° and proceed with DNA extraction the next day.

Milk production measurement

The animals were milked once a month until the end of the productive season, and the amount of milk was measured in the field using a cylinder, milk samples for each animal were placed in special tubes and transferred in a refrigerated storage box to the laboratory. The proportions of milk components (solids, fat, protein, lactose) were measured, and the length of the milk season was calculated.

DNA Extraction

DNA was extracted from blood according to the instructions of the diagnostic kit supplied by the company ReliaPerpTM Blood gDNA Miniprep system, Promega, and the concentration of the extracted DNA was measured in order to determine the quality of the samples using a Quantus Fluorometer to detect the concentration of the extracted DNA, by Add 1 µl of extracted DNA to 199 µl of diluted Quanti Fluor dye and mix well, then the mixture was placed at room temperature for 5 minutes, and then the DNA concentration values were measured and were 8.9 ng/ml. DNA purity was measured using a Nanodrop device, and this device detects the potential error rate in the sample if there is contamination in the sample if it contains protein or other substances, as the standard reading of DNA is equal to 1.8 and if the readings differ from this percentage, it is an indication of the presence Contamination in the sample, the reading was taken at a wavelength of 260-280 nm.

Molecular characterization of the studied gene

One region of the FASN gene was identified that contains three exons of this gene, and the primer was designed for the studied region based on the NCBI website for the purpose of conducting molecular detection and knowing the polymorphisms of the genes and the mutations in them, as shown below:

Table (1): Primer pair used in this study

Primer Name	Seq.	Annealing Temp (OC)	Product size (bp)
FASN – F	**5'- CTCATGTCCAGGGTACAATAC -3'**	**60**	987
FASN – R	**5'- GAACCGTCAAACAGGAAGA -3'**		

The primers were provided by the Korean company Macrogen in the form of a lyophilized powder, and Al-Bawadi was dissolved by cooling by adding 300 microliters of deionized water for the purpose of obtaining the final required concentration of 100 pmol/µl as a primer stock solution. Preparing the working solution for these initiators

by adding 10 µl of stock solution (stocked at -20 °C) to 90 µl of deionized water to obtain the final concentration of the working solution which is 10 pmol/µl.

Polymerase chain reaction (PCR) of the studied gene

Molecular detection of the studied gene (FASN) was carried out and the DNA copies were amplified using the Polymerase chain reaction (PCR) and using the GoTaq Green Master Mix diagnostic kit with a volume of 25 microliters. Polymerase reaction according to the reaction conditions of each duplicated gene segment, and after the completion of the reaction, the reaction product was transferred to the electrophoresis device to ensure that the required piece of DNA and the materials used in the molecular detection were doubled. Total volume 25 µl (Primer for forward and revers 1 µl - Master mix 12.5 µl – Nuclease Free Water 7.5 µl – DNA 3 µl).

The program used in the molecular study of the studied gene

The program used for molecular detection using PCR technology was applied, starting with the Initial Denaturation stage at a temperature of 95 °C for a period of 5 minutes with a number of cycles (1), and in the stage of Denaturation at a temperature of 95 °C, and Annealing at 60 °C, and Extension 72 °C for a period of 30 seconds and 30 cycles, and the Final Extension stage 72°C for 7 minutes, Hold phase 10°C for 10 minutes, and cycles (1).

Molecular characterization of the phenotypic polymorphism of FASN gene using DNA sequencing technique

The PCR product in a volume of 20 microliters was sent to the Korean Macrogen Corporation - Korea for the purpose of reading the sequences of nitrogenous bases and detecting genetic mutations in them. nih.go The nucleotide sequence profile was used to determine the presence or absence of the mutation and the curve profile to determine the phenotypic polymorphism of the FASN gene.

Statistical analysis

The data were statistically analyzed using the Statistical Analysis System–SAS program [7] to study the effect of FASN gene polymorphism on the studied traits according to the mathematical model, and the significant differences between the means were compared using Duncan (1955) multinomial test by applying the Least square means method.

The Chi-square-$\chi 2$ test was also used to compare the percentages of the distribution of genotypes for each gene in the sheep sample.

Results and Discussion

Gene extraction Fatty acid synthase (FASN)

The FASN gene was extracted using PCR technique, the PCR product was electrophoresed on an agarose gel at a concentration of 1.5% and the migration product was photographed to ensure the success of the gene extraction process and to obtain the required gene at a size of 987 bp using the Ladder DNA Marker (100 bp) Figure (1).

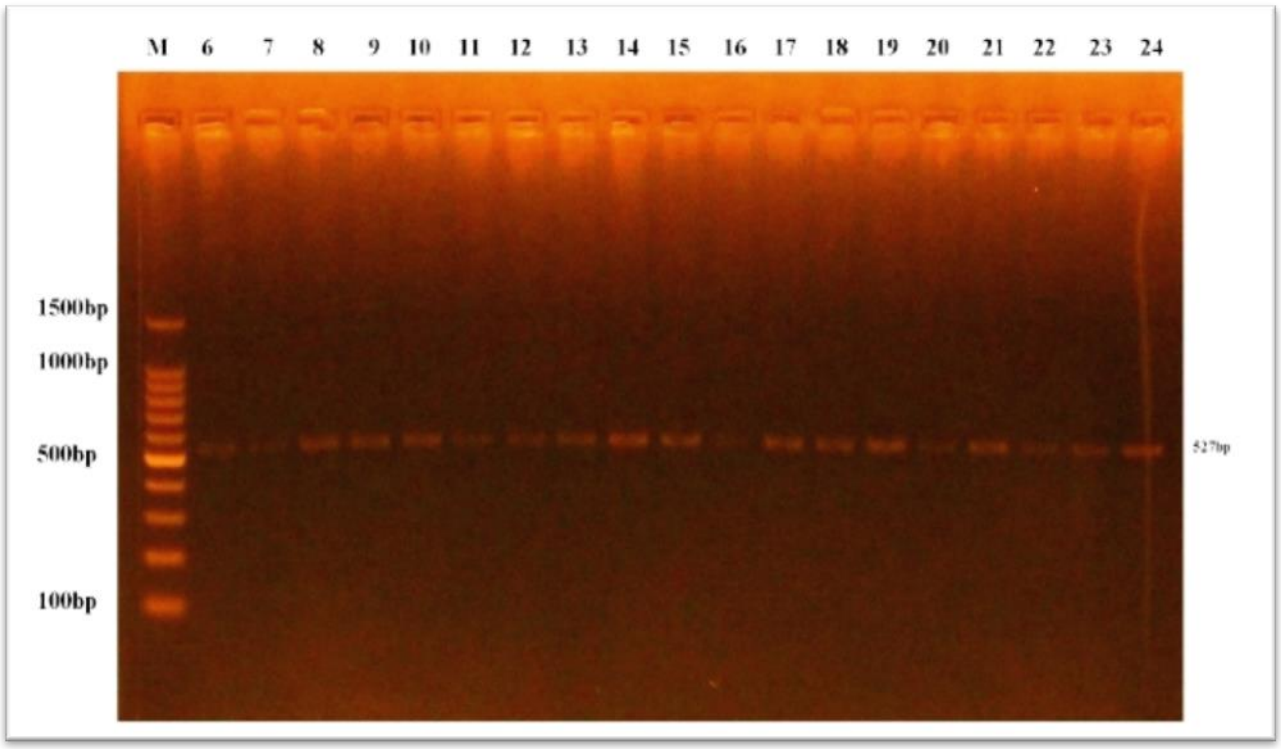

Figure (1): Extraction of the FASN gene on agarose gel at a concentration of 1.5%

The sequences of the nitrogenous bases of the studied gene FASN

Nitrogenous base sequencing technology was used, and the results of the study showed that the length of the studied segment of the FASN gene is 987 base pairs and contains three pieces, namely exons (39, 38, 40) starting with a length of 469 bp and ending with 1456 bp, and one mutation was detected in the region Exon (38) is (A2253C) with sequence rs161102729, and individuals appeared with three genotypes: wild AA, hybrid AC and mutated CC, and through this mutation, the A allele was replaced by the C allele. Figure (2).

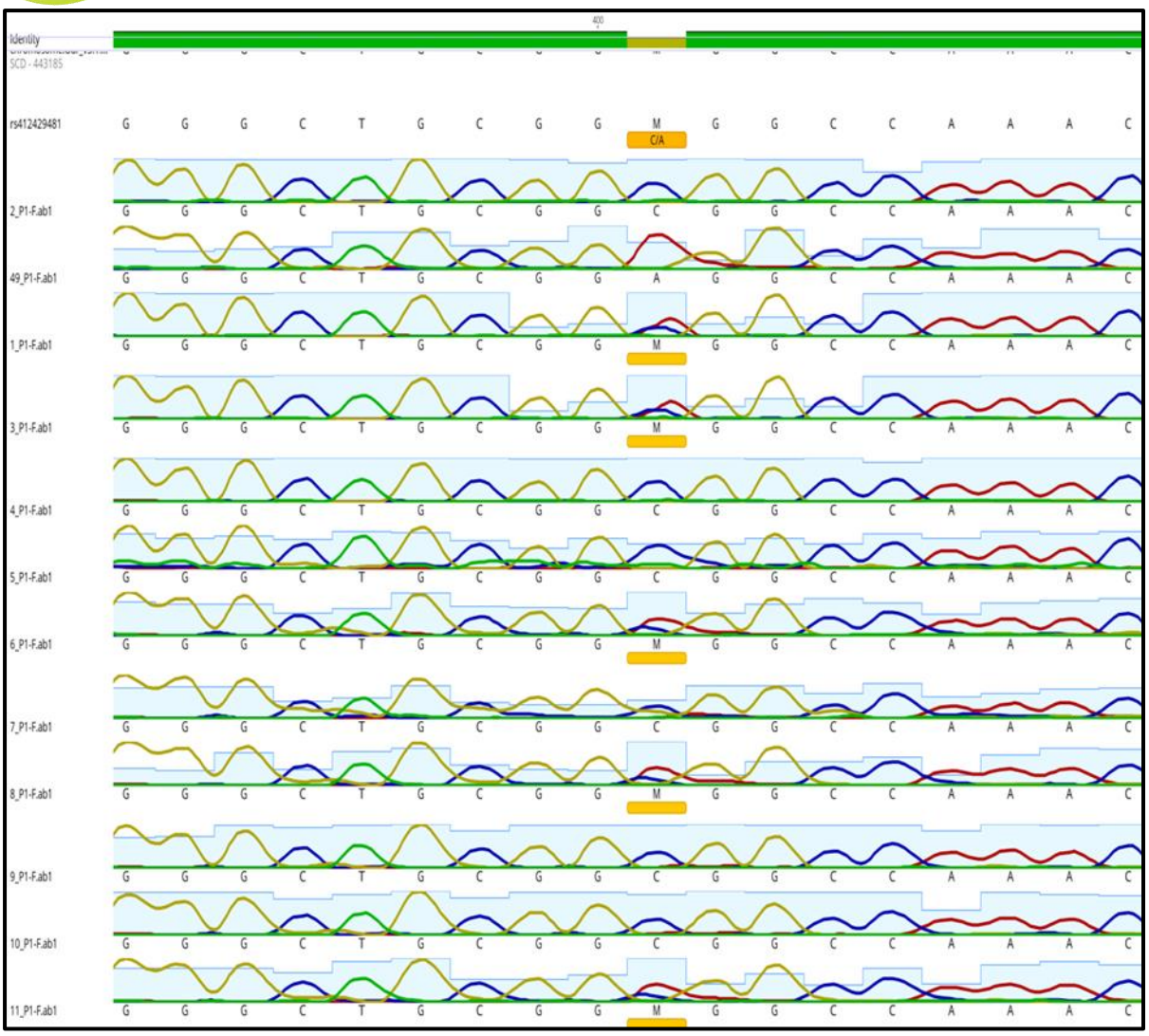

Figure (2): The site of the mutation in the gene

Number, percentages of genotypes and allelic repeats of FASN gene in Awassi sheep.

It is noted from (Table 1) the number and percentages of genotype for the FASN gene, as significant differences (P≤0.01) appear between the distribution ratios of these different genotype, which amounted to 7.87, 21.57 and 70.59% for each of the AA, AC and CC genotype, respectively, and through These percentage show that the percentage of animals with the mutant CC genotype are higher than the numbers with the heterozygous CA genotype, followed by the wild genotype AA, through the analysis of the FASN gene for local Awassi ewes, according to a study conducted by [8], on the Hogan and Long-tailed Than sheep breed. It was found that the distribution of animals with the wild AA genotype was 14%, the AC hybrid was 54%, while the CC mutant genotype was 32%, and in another study conducted by [9], on the Cypriot Chios sheep breed, it was found That the distribution of animals with the mutated TT

genotype was 41%, which is higher than the two genotype CC and CT 34 and 25%, respectively, and in a study [10] conducted on the Polish *Zošľachtená Valaška* sheep, it showed only two genotype, the wild CC, reached 56.18% and the Turk The CT heterozygous showed 43.82%, that the differences in the studies may be due to the presence of differences between breeds and nutrition, and other genetic factors such as the effect of modified genes [11]. Sometimes it is to tailor the environment to another (its suitability to the environment) or as a result of breeders detailing a certain level of the trait's appearance, and then this is reflected in the proportions of the distribution of genotypes, and this relative difference is also due to the size of the studied sample [12].

It is clear from Table (2) that three genotypes appeared, namely AA, AC and CC, responsible for the two alleles, A and C. The allele frequency A of the FASN gene in the local Awassi sheep sample studied was 0.19, while the frequency of the C allele was 0.81, and this The result reflects a clear presence of the C allele of the FASN gene in Awassi sheep. The reason may be attributed to the type of breeding adopted in the station and the sample size, or to the adaptation of the wild allele to environmental factors, and this result was identical to what was stated in the results of the study [8]. The results of a study [13] on the *Altamurana* sheep breed, where the allelic frequency was 0.93 and 0.07 for each of the C and T alleles, respectively.

Table (2): Distribution of FASN gene polymorphism and allele frequency in Awassi Sheep

Genotype	Number	Percentage (%)
Wild: AA	4	7.87
Hetero: AC	11	21.57
Mutant: CC	36	70.59
Total	51	100 %
Chi-square value ($\chi 2$)	----	56.647 **
Allele	Frequency	
A	0.19	
C	0.81	
Chi-square value ($\chi 2$)	14.302 **	
(P≤0.01)**		

Effect of FASN gene polymorphisms on milk production and Lactation period in Awassi sheep

The results showed a significant difference (P≤0.05) in the total milk production among ewes according to the FASN gene polymorphism, as individuals carrying the genotype AA outperformed the other individuals carrying the CC and AC genotypes,

and the amount of milk produced was 262.50, 233.33 and 218.18 kg, respectively. (Table 3), and there were no significant differences between AA, AC and CC genotype in Lactation period, which means that the differences in the amount of milk produced are due to the increase in daily production of individuals carrying the genotype AA and then CC.

By selecting individuals carrying the AA appearance, it is possible to improve the milk production characteristic of Awassi sheep, and daily milk production is one of the important economic characteristics that have a positive and moral correlation with the total production and its necessary role in the growth of newborns during the lactation period. This result agrees with what other studies have indicated in terms of the significant effect of the FASN gene polymorphism on milk production [7], and in contrast with some others in that the amount of milk production in their studies was less than the amount of total milk production in the current study [14], [15]. This difference may be due to non-genetic factors such as the number of milking times, season, stage of lactation and feeding, as well as due to genetic factors such as polymorphisms in the coding and non-coding regions. [13].

The discrepancy in the results of the studies indicates the presence of overlaps between the FASN alleles and the occurrence of genetic mutations, as well as the difference in the number of observations with different genetic manifestations of this gene. Increasing the number of samples and for different herds and studying more than one plot for the same gene would give more accurate results due to the presence of differences in Genetic diversity between local and foreign breeds, as well as differences in management and production systems, led to a genetic regression in the characteristics of milk production in all farm animals, so many researchers and studies focused on the importance of genetics and finding modern and developing methods for genetic improvement processes through knowledge of the effects of genes and parameters Genetics and genetic manifestations [16], and their effective role in the production of milk and its components of protein and fat, especially the multiplicity of genetic manifestations of milk proteins such as caseinate and lactoglobulin. Several European countries emphasized the importance of this issue, which led to an improvement in the milk production rate from 1-2 % annually for sheep [17].

Table (3): The relationship of the FASN gene genotype to milk production and Lactation period in Awassi sheep

Genotype	Number	Mean ± standard error	
		Total milk production (kg)	Lactation period (day)
Wild: AA	4	262.50 ±19.84 a	101.75 ±11.19
Hetero: AC	11	218.18 ±22.04 b	102.36 ± 4.03
Mutant: CC	36	233.33 ±26.52 b	106.36 ±4.62
Morale level	---	**	NS
The averages with different letters within the same column differ significantly between them. (P≤0.01)** . NS: insignificant			

Effect of polymorphisms of FASN gene on milk components of Awassi Sheep

Table(4) shows the results obtained through the statistical analysis of the polymorphisms of the FASN gene, indicating that there were significant differences (P≤0.05)) in the percentage of protein according to the different genotype of the FASN gene, as the AA genotype was superior to the highest percentage of protein and amounted to 6.27% compared to According to the two genotype AC and CC, which amounted to 5.13 and 5.83%, respectively, this result is similar to what was found by [10], [7], in terms of the emergence of highly significant results in the proportion of protein according to the genotype in the Spanish Shura sheep, the French Lacon and the Czech sheep, And by observing the effect of the FASN gene (Table 3), on the proportions of some other milk components represented in the percentage of fat, lactose and non-fat solids, it is clear that they did not differ significantly according to the different genotypes of this gene, and these results matched with what was reached [18], [19].

It is clear from the results that there is an inverse relationship between total milk production and its components, which means that the genotype of the expressed genes for the production of large quantities of milk do not necessarily have the same effectiveness in expressing the components of milk. Between the increase in milk production and the proportions of its components in sheep [20], the existence of differences between the proportions of milk components can be due to the difference in the breed, the method of analyzing these components, and the difference in environmental conditions between the above research.

The protein and fat content of Tibetan sheep milk is 4.84 and 6.94%, respectively [21], and the protein content of sheep milk was determined by [22] 6.35, fat 6.90, lactose 5.00, and dry matter 19.03%, and the protein content in our current study was within the existing range of values. On the other hand, while the other milk

components in terms of fat, lactose and solids were much less, and this may be related to the difference in keeping animals, feeding them, lactation stage, age, health status and other things [23], [24].

Table (4): The relationship of FASN gene polymorphism to the components of milk in Awassi Sheep.

Genotype	Number	Mean ± standard error			
		Fat (%)	Lactose (%)	Protein (%)	Non-fat solids (%)
Wild: AA	4(12sample)	3.75 ±0.75	3.92 ±0.39	6.27 ±0.22a	12.42 ±0.61
Hetero: AC	11(33sample)	4.10 ±0.30	4.21 ±0.32	5.13±0.45b	11.64 ±0.49
Mutant: CC	36(108sample)	4.13 ±0.18	4.01 ±0.15	5.83 ±0.21a	11.66 ±0.31
Morale level	---	NS	NS	*	NS
The averages with different letters within the same column differ significantly between them. (P≤0.05)* ،NS: insignificant.					

The protein and fat content of Tibetan sheep milk was 4.84 and 6.94%, respectively, and the protein content of sheep milk was determined as 6.35%, fat 6.90%, lactose 5.00%, and dry matter 19.3% [6;9], In this study, the level of protein and lactose in The milk was within the range of values found above, while the fat and dry matter content was much lower, which may be related to the difference in keeping and feeding the animals in infancy, age, feeding and health status [5].

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