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Original Article

Relationship between *SCD1* Gene Polymorphism and the Production and Composition of Milk in the Iraqi Awassi Sheep

Naeemah, A. G[1 *], Al-Anbari, N. N[2]

1. Republic of Iraq-Ministry of Agriculture, Office Planning and Follow–Up, Animal Production Division, Iraq
2. Animal Production Department, College of Agricultural Engineering Sciences, University of Baghdad, Baghdad, Iraq

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Corresponding Author: aliggnnr@yahoo.com

Abstract

Selection based on genetic makeup became an important tool in genetic improvement. The development of molecular biology opened the way to study the genes of farm animals and genetically improve them. The aim of this study was to determine the Allele frequency and genotype distribution of the *SCD1* gene and its relationship to milk production and its main components from fat, protein, lactose and non-fat solids percentage in Iraqi Awassi sheep. Fifty-one female Awassi sheep were used in this study. The genotype distribution of the *SCD1* gene in the studied Awassi sheep sample was 50.98, 41.18 and 7.84% for each of the CC, CA and AA genotypes, and the discrepancy between these percentages were highly significant ($P \leq 0.01$), and the appearance of the C and A alleles with a frequency of 0.72 and 0.28 respectively, highly significant differences ($P \leq 0.01$) appeared in the total milk production according to the genotype. As for the milk components, it was found that there were significant ($P \leq 0.05$) differences in the percentage of fat and the percentage of non-fat solids. From the results of the current study, it can be concluded that the *SCD1* gene can be adopted as an important indicator in developing 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 product performance.

Keywords: *SCD1* gene, genotype, milk production and components

1. Introduction

Selection based on genetic makeup became an important tool in genetic improvement. The development of molecular biology opened the way to study the genes of farm animals and genetically improve them (1). Further improving the capabilities of scientists in modifying the genomes of cows and sheep with desired traits lead to improving the productivity of agricultural animals (2). In addition, many aspects of animal genome projects have contributed to revolutionizing the genetic analysis of living organisms through DNA, allowing direct identification and manipulation of DNA sequences and applying MAS-assisted selection to complement the traditional selection methods (3). The dairy industry is important for the sheep breeding program because of its high content of fat, protein, lactose, ash, and total non-fat solids, which have a high nutritional value (4). Sheep milk can also be processed into high-quality dairy products such as cheese and milk powder, achieving high economic interest with the increasing interest in sheep's milk, whose content has become more clear; it was found that the nutritional value of sheep's milk is higher than that of goat and cow's milk with abundant fat production, protein and mineral contents, which provide a good opportunity for the development of sheep milk industry (5). Research into the genetic factors of sheep milk has made some progress. Recent studies have emerged to detect genes that affect the growth of the mammary

gland, such as the Stearoyl-CoA desaturase (SCD) gene, which greatly affects milk production and composition characteristics (6). That gene expression The SCD gene is mainly found in the mammary gland of farm animals, including sheep (7). The current study aimed to determine the relationship between genetic phenotypes of the *SCD1* gene to milk production and its main components in a sample of Iraqi Awassi sheep.

2. Materials and Methods

The study included biochemical and genetic aspects. 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 analyses were conducted in the laboratories of scientific progress specialized in molecular genetics and biotechnologies, aiming to separate the genetic material and determine the Phenotype of the *SCD1* gene.

2.1. Animals

Fifty-one female Awassi sheep were used in this study, 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.

2.2. 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.

2.3. 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 milking season was calculated.

2.4. 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 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. 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. If the readings differ from this percentage, it indicates the presence of contamination in the sample, and the reading was taken at a wavelength of 260-280 nm.

2.5. Molecular Characterization of the Studied Gene

The *SCD1* gene was identified, and the primer (8, 9) was determined for conducting molecular detection and knowing the polymorphisms of the gene and the mutations present in it, as shown in table 1.

Table 1. Primer sequence of *SCD1* gene

Primer Name	Seq.	Annealing Temp (^{O}C)	Product size (bp)
SCD1-F	5'- AAATTCCCTTCGGCCAATGAC -3.'	60	527
SCD1-R	5'- TCTCACCTCCTCTTGCAGCAA-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 to obtain 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.

2.6. Polymerase Chain Reaction (PCR) of the Studied Gene

Molecular detection of the studied gene (*SCD1*) was carried out, and the DNA copies were amplified using the Polymerase chain reaction (PCR) and 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 reverse 1 µl - Master mix 12.5 µl – Nuclease Free Water 7.5 µl – DNA 3 µl).

2.7. 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 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".

2.8. Molecular Characterization of the Phenotypic Polymorphism of *SCD1* Gene Using DNA Sequencing Technique.

The PCR product in a volume of 20 microliters was sent to the Korean Macrogen Corporation - Korea to read the sequences of nitrogenous bases and detect 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 *SCD1* gene.

2.9. Statistical Analysis

The data were statistically analyzed using the Statistical Analysis System–SAS program (Version 9.1) to study the effect of *SCD1* 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.

Mathematical model: the relationship of *SCD1* gene polymorphism to the studied traits:

Yijklm = µ + *SCD1*i + Aj + Sk + Ol + eijklm

Yijklm: observational value m due to genotype i, mother's age j, newborn's sex k, and birth month l. µ: the general average of the adjective. *SCD1*i: influence of *SCD1* gene polymorphism (CC, CA, AA). Aj: effect of ewe age (4, 5, 6 years). Sk: the effect of the gender of the newborn (male, female). eijkl: the naturally distributed random error with a mean of zero and a variance of σSe.

3. Results and Discussion

3.1. Gene Extraction Stearoyl-CoA Desaturase-1 (*SCD1*)

The *SCD* gene 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 527 bp using the Ladder DNA Marker (100 bp) (Figure 1).

3.2. Nitrogenous Base Sequences of the Studied Gene *SCD1*

Nitrogenous base sequencing technology was used, and the study results showed that the segment length is 527 base pairs, and one mutation was detected in the Promoter region, which is C-211A with the sequence rs412429481. The individuals appeared with three genotypes: wild CC, hybrid CA and mutant AA. C by the A allele, figure 2.

3.3. Number, Percentages of Genotypes and Allelic Repeats of *SCD1* Gene in Awassi Sheep

It is clear from (Table 2) the number of animals and their genotypes and the percentage distributed over the studied sample. It shows significant differences between the percentage of the different genotypes, which amounted to 50.98, 41.18 and 7.84% for structures CC, CA and AA, respectively. These percentages show that the percentage of animals with The wild CC genotype is higher than the numbers with the CA heterozygous genotype, and a small percentage of animals bearing the AA mutant genotype appeared. These results differed from the results of some previous studies, as it came in the study of the CA structure in the foreground (10), followed by the mutant genotype. AA and then the wild CC on a Polish sheep breed (CA 54%, CC 36%, AA 10%) and in a study (11) on Iranian sheep, the wild CC overcame the heterozygous CA (CC 59%, CA 41%) and in Another study (12) (CA 59%, AA 28%, CC 13%) the distribution of a percentage of genotype certainly differs if compared between different breeds, as this relative difference is due to the difference in the breed and its adaptation to environmental conditions, as well as to the size of the studied sample.

The frequency of the wild allele ((C) was 0.72 and 0.28 for the mutant allele (A), meaning that there is a commonness for the wild allele C at the expense of the mutant allele A in the sample of Awassi sheep that were studied (Table 2), and this may be attributed to the type of breeding adopted in the station. The size of the station the sample or the adaptation of the wild allele to environmental factors, and this result came close to the results of the study of Kaplanova, Dufek (12), where the allelic frequency was 0.58 and 0.42 for the C and A alleles, respectively. It differed in the study of Inostroza, Scheuermann (13), where the allelic frequency appeared to be 0.38 for the A allele and 0.62 for allele C.

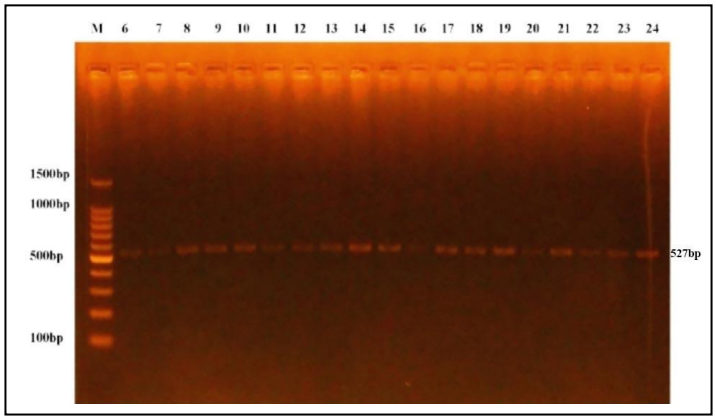

Figure 1. Extraction of the *SCD1* gene on agarose gel at a concentration of 1.5%

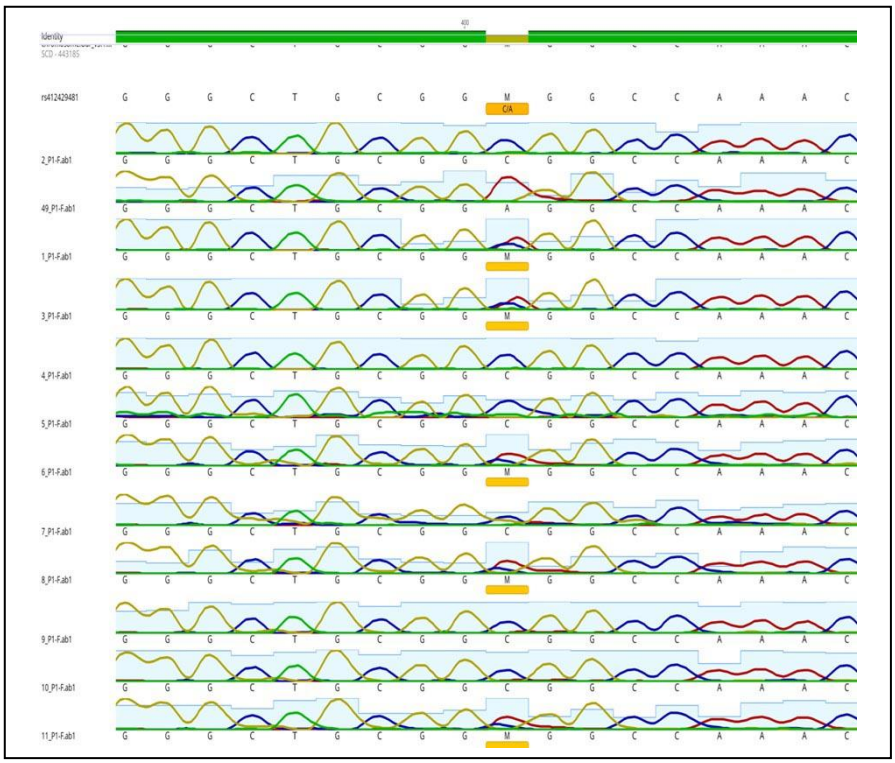

Figure 2. The site of the mutation in the *SCD1* gene

Table 2. Distribution of *SCD1* gene polymorphism and allele frequency in Awassi Sheep

Genotype	Number	Percentage (%)
Wild: CC	26	50.98
Hetero: CA	21	41.18
Mutant: AA	4	7.84
Total	51	100 %
Chi-square value ($\chi 2$)	----	20.568 **
Allele	**Frequency**	
C	0.72	
A	0.28	

($P \leq 0.01$)**

3.4. Effect of *SCD1* Gene Polymorphisms on Milk Production and Lactation Period in Awassi Sheep

The results of the current study showed that there were highly significant differences ($P \leq 0.01$) in the total milk production according to *SCD1* gene polymorphism, as the total rate of milk production reached the maximum among ewes with the genotype AA (300,000 kg), while the total production of the genotype CA reached an average of 252.38 kg and was below at the genotype CC at 205.77 kg (Table 3). This result makes it possible to improve the milk production characteristic of Awassi sheep through selection for individuals carrying the AA phenotype. Milk production is one of the important economic characteristics for its necessary role in the growth of newborns during the lactation period. There were no significant

differences between the genotype of the multiple effects of the *SCD1* gene in the Lactation period, as its averages were 104.26, 104.85 and 112.25 days for the three genotypes CC, CA and AA, respectively (Table 3).

The discrepancy in the results of the studies indicates the presence of overlaps between the *SCD1* alleles and the occurrence of genetic mutations, as well as the difference in the number of observations with different genetic manifestations of this gene, and that 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 breeds, as well as differences in management and production systems, led to a genetic decline in the characteristics of milk production in all farm animals, including sheep, 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 genetic parameters and genetic manifestations (14) and their effective role on milk production and its components of protein and fat, especially the multiple 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 annual milk production rate from 1-2 % for sheep and goats (15).

3.5. Effect of Polymorphisms of *SCD1* Gene on Milk Components of Awassi Sheep

Table 4 shows that the differences in the percentage of protein and lactose in milk according to *SCD1* gene polymorphism were not significant for Awassi ewes with genotypes CC, CA and AA. The percentage of protein and lactose by the different *SCD1* gene polymorphisms in sheep. By noting the results in table 4, it is clear that the significant ($P \leq 0.05$) effect of the *SCD1* gene on the fat percentage in the milk was reached, as the percentage reached a maximum of 4.29%) in the milk of mothers with a genetic profile. CC and then the genotype CA, amounted to 3.92%, while it was the lowest (3.75%) in the similar ones with the genotype AA, which is consistent with the findings of some studies (8, 10). *SCD1* in the percentage of solids according to the different genotypes of CC, CA and AA, which amounted to 11.53, 11.61, and 13.46%, respectively, and it was noted that there was an effect of polymorphism in the gene encoding β-lactoglobulin proteins on milk formation (16, 17), as it was found that there is a Genetic link variability between β-lactoglobulin proteins and milk protein and fat content in Awassi sheep and Morkarman sheep, and there was no relationship with milk solid components, acidity and milk coagulation (18). Fat percentage is one of the most important structural characteristics of milk that determines the quality of milk, its price and the type of product from which it is made. Therefore, the adoption of gene expression in improving this characteristic appears feasible through the results of this study. It was noted that the presence of some alleles in the animal genome could negatively affect On the percentage of milk fat; some alleles increase and others decrease the percentage and also affect the components of milk and the health of the udder (19), and there is an inverse relationship between the amount of milk produced and the percentage of fat in milk, and therefore the excellence in the production of whole milk referred to above (Table 3) in Awassi ewes Local sweeteners with AA genotype had the lowest fat content (Table 4). No research was found on the effect of genetic makeup on polymorphisms in the *SCD1* genes on the composition and ratio of protein types in sheep's milk and the level of protein or lactate.

Table 3. The relationship of the *SCD1* gene genotype to milk production and Lactation period in Awassi sheep

Genotype	Number	Mean±standard error	
		Total milk production (kg)	Lactation period (day)
Wild: CC	26	205.77±22.33^{c}	104.26±5.25
Hetero: CA	21	252.38±18.76^{b}	104.85±5.32
Mutant: AA	4	300.00±27.26^{a}	112.25±5.54
Morale level	---	**	NS

The averages with different letters within the same column differ significantly between them. ($P\leq0.01$)** . NS: insignificant

Table 4. The relationship of *SCD1* gene polymorphism to the milk components in Awassi Sheep

Genotype	Number	Mean±standard error			
		Fat (%)	Lactose (%)	Protein (%)	Non-fat solids (%)
Wild: CC	26(78sample)	4.29±0.20^{a}	4.13±0.18	5.77±0.26	11.53±0.28^{b}
Hetero: CA	21(63sample)	3.92±0.26^{ab}	3.99±0.22	5.72±0.30	11.61±0.45^{b}
Mutant: AA	4(12sample)	3.75±0.62^{b}	3.80±0.57	5.31±0.57	13.46±0.65^{a}
Morale level	---	*	NS	NS	*

The averages with different letters within the same column differ significantly between them. ($P\leq0.05$)* ،NS: insignificant

Tibetan sheep milk's protein and fat contents were 4.84 and 6.94%, respectively. The protein content of sheep milk was determined as 6.35%, fat 6.90%, lactose 5.00%, and dry matter 19.3% (20, 21); in this study, the level of protein and lactose in the milk was within the range of values found above. In contrast, 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 (10).

Authors' Contribution

Study concept and design: A. G. N.
Acquisition of data: A. G. N.
Analysis and interpretation of data: N. N. A.
Drafting of the manuscript: N. N. A.
Critical revision of the manuscript for important intellectual content: A. G. N. and N. N. A.
Statistical analysis: A. G. N.
Administrative, technical, and material support: A. G. N.

Ethics

The study protocol was approved by the ethics committee of the Republic of Iraq-Ministry of Agriculture, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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