Some Genetic Manifestations of the SCD1 Gene and its Relationship to some Productive and Reproductive Characteristics of Iraqi Local Goats

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Abstract
This study was conducted with the aim of identifying some manifestations genetic parameters of the SCD gene and its relationship to some productive characteristics of local goats, studying the components of milk from protein, fat, lactose and non-protein mineral elements, estimating the live weight of mothers, newborn weight, weaning weight, milk season length and nocturnal repetition, the effect of live weight was significant ($P<0.05$) in all genetic manifestations as well as the length of the milk season is significant ($P<0.05$) in most genetic manifestations and the percentage of fat in the components of milk was also significant ($P<0.05$), other productive qualities and milk ingredients had no significant effect.
Keywords: SCD1 Gene; Iraqi Local Goats; Genetic Manifestations

1. Introduction

Food is the most important factor in the growth and development of the population and cannot be dispensed with because it contains basic components such as protein, carbohydrates, fats, vitamins, mineral elements and others, animal production is one of its basic components and is the first source of providing these ingredients through meat and milk of various types of animals (cows, buffaloes, sheep and goats) in addition to some other animals. Reproductive traits, growth qualities, milk production and fertility are of great importance in the goat industry, however, these traits are difficult to improve by traditional selection because it is difficult to identify genes that are directly related, responsible and controlling these traits, however, the introduction of molecular genetics techniques has contributed significantly to overcoming these difficulties and providing new opportunities to improve such traits by adding tools to identify genetic changes at the DNA level as well as identifying the genes responsible for these traits individually (Sonika et al., 2015).

The traditional selection of breeds of agricultural animals, including cows and buffaloes, depends on morphological traits, and in the last century the quantitative theory was adopted in order to improve the selection processes and predict genetic responses to the improvement processes, this led to the selection of a number of genetic traits of economic importance in farm animals, selection based on genetic makeup has become an important tool in the process of genetic improvement of farm animals (Liron and Givambatt). 2006) Biological evolution, the discovery of genetic maps and molecular genetics have led to the identification of many means and programs that will improve the performance of animals, the discovery of polymerase change reaction (PCR) technology has transformed the course of biological sciences, with its impact extending to countless subdisciplines of biology (Lorenz, 2012). Scientists have invested this technique to study and track genetic mutations that occur on the genetic material of living organisms, including Single Nucleotide Polymorphisms (SNPs) and use them as genetic markers on the basis of which selection is made, especially for productive traits with low genetic equivalent that are controlled by a number of genetic sites, which are known as quantitative traits sites (Quantitative traits loci-QTL), through these sites and the knowledge of the associated markers, it is possible to predict the phenotypic variation of the qualities to be improved early and develop selection programs based on them (Williams, 2005).

Local Goats

This goat is spread throughout Iraq, where it is found in the desert and countryside and near cities and the predominant color is black and there are other colors, including white and brown, and we may find individuals all in one color, such as brown or gray, also find different qualities between individuals, such as the presence or absence of horns, small or larger ears, small or large size, and all these result from confusion between individuals and herds and is characterized by coarse hair with a length of 10-15 cm.

The weight of the hair clip reaches 2.5 kg and wool is used in the manufacture of carpets, the average weight of the animal is 30 kg produced from medium milk and meat is of acceptable quality and quantity (AL-Saigh and AL-Kass (1992).

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2. Materials and Methods of Work

This study was conducted at the Ruminant Research Station of the General Organization for Agricultural Research / Ministry of Agriculture in Abu Ghraib on a sample of 30 goats of local species, while laboratory analyzes were carried out in the laboratories of the Scientific Progress Company specialized in molecular genetics and biotechnology, with the aim of separating the genetic material and determining the genetic structures (Genotype) of the SCD1 gene and its relationship to the productive and reproductive performance and growth characteristics of local goats.

Herd Management

The animals are raised in semi-open pens (40% roofed and 60% open) designated for their shelter, the herd is managed according to a program that includes nutrition, preparation for the mating season, preparation for pregnancy and childbirth, as well as health and veterinary care.

Nutrition

Nutrition animals at the station depends on grazing, as they are taken out for grazing in the winter from 8 am to 2 pm, and in the summer they are taken out for grazing from 8 am to 12 pm and from 3 pm to 6 pm. Upon her return to the station, her food needs are supplemented by providing jet, barley and alfalfa mixture, the method of food payment was used a month before the start of feeding, where animals are given 750 grams of concentrated feed per head per day, "and the amount is adjusted after feeding to 500 grams per head per day." The concentrated feed consists of the following:

Ingredients	Diet 1	Diet 2	Diet 3
Barley grains	42	40	25
Wheat bran	45	42	30
Soybean meal	_	_	12
Yellow corn	10	15	30
Calcify	2	2	2
Table salt	1	1	1

Mating season

Mating often begins at the station at the end of August for two months and ends at the end of October, the introduction of goats known numbers and record their pedigree to the herd of goats and continue until the end of the two periods of estrus, and after the end of the estrus season is the introduction of goats scouts to ensure mating of all goats, after that, the date of mating is recorded, the goat number, the weight of the goat when mating, the goat is weighed at birth, the newborns are weighed and numbered 24 hours after birth, and the colostrum is given from the first hour of birth, and the newborn continues to breastfeed until weaning.

Veterinary & Healthcare

The station's animals are subject to a health and preventive program according to the following steps:

1- Dipping animals to eliminate external parasites with a solution of pythroid cypromethrin at a concentration of 10% four times a year (May, June, September, October).
2- Vaccinate animals with enterotoxaemia vaccine twice a year.
3- Vaccination against Brucella and Foot-and-mouth Disease (FMD).
4- Dosing animals with Findex to prevent liver and intestinal worms twice a year
5- Treatment of mastitis in case of occurrence.
6- Vaccinate animals annually to protect against PPR.
7- Spraying sheds with pesticides and disinfectants for parasite control.

Field Measurements

Blood Samples

3 ml of blood was collected from the jugular vein area of each animal in a collection tube containing an EDTA K3 anticoagulant and the animal's number was recorded in each tube of its own and transported in a dedicated refrigerant storage box to the laboratory and kept in the refrigerator at a temperature of 4°C and the start of DNA extraction the next day.

Measuring Milk Production

Milk production was recorded once a month until the end of the production season, and the amount of milk in the field was measured using a graduated cylinder (Cylinder), as the newborns were isolated from their mothers in the evening and milked the next morning early and then the newborns were released to the mothers, milk samples were taken from each animal and milk samples were placed for each animal in special tubes and transported in a refrigerated storage box to the laboratory and the proportions of milk components (total fatty acids, non-fatty solids, fat percentage, protein, lactose were measured), the length of the milk season was calculated, and the peak production was calculated, and the total milk production was calculated from the following equation:

Total Milk Production = daily milk production rate × number of milking days

Calculation of fertility rate at birth

The fertility rate is defined as the number of births in one abdomen or the number of births resulting from one vaccination and is calculated as in the following equation:

Fertility Ratio = Number of Births Born / Number of Mothers (AL-Saigh and AL-Kass (1992)

Laboratory Work

DNA extraction method

DNA was extracted from the blood according to the instructions of the diagnostic kit prepared by ReliaPerp ™ Blood gDNA Miniprep system,

Promega, and according to the following:

1- The blood sample was mixed well for 15 minutes at room temperature using a Roll Mixer vibrator.

2- In each tube of 1.5 ml 20 µl of Proteinase K (PK) solution was placed, then 200 µL of blood sample was added to it and mixed manually and carefully for 10 seconds.

3- 200 µL of Cell Lysis Buffer (CLD) solution was added to each tube and mixed with a Vortex device for 10 seconds.

4- The tubes containing the mixture were placed in a water bath with a temperature of 56 ° C for 30 minutes.

5- During the incubation of the mixture samples in the water bath, The ReliaPrep™ Binding Column was placed with the filter was prepared and placed in the collection tubes designated for the centrifuge.

6- The pipes were removed from the water bath and 250 µL of Binding Buffer (BBA) were added to them and mixed by the Vortex device for 10 seconds.

7- All the contents of each tube were transferred to the ReliaPrep ™ Binding Column tubes and placed in the centrifuge for 3 minutes at 12,000 rpm.

8- After the end of the centrifugation process, the collection tubes containing the clear solution were destroyed and the remaining sediment in the ReliaPrep™ Binding Column tubes was retained and placed in new collection tubes.

9- 500 microliters of Column Wash Solution (CWD) were added to each tube and placed in the centrifuge for 3 minutes at full speed, then the clear was eliminated and this step was repeated three times.

10- After the washing step, the tubes containing the sediment were placed in micro tubes dedicated to centrifugation with a capacity of 1.5 ml and 100 microliters of Nuclease-Free Water were added to them and placed in the centrifuge for 5 minutes at a speed of 5000 rpm.

11- After that, the tubes containing the filter The ReliaPrep™ Binding Column were disposed of and the clear solution containing the DNA genetic material was kept.

Gene SCD1

It is an endoplasmic reticulated enzyme that works to reduce the rate of formation of monounsaturated fatty acids (MUFAs), specifically oleic acid and palmitoleate acid from the enzyme stearol, these two acids are the main components of membrane phospholipids and cholesterol esters, which are the main components of triglycerides in humans, and the enzyme is encoded by the gene SCD (Calvo et al. (2019).

It has an important role in the formation of fatty acids in animal cells in milk (Griinari et al. (2000) and adipose tissue AT (Daniel et al. (2004) An enzyme encoded by SCD contains iron and is catalyzed to reduce the rate of synthesis of unsaturated fatty acids, and the main product of SCD is oleic acid, which consists of fatty acid desaturation.

In the human genome SCD is expressed largely in adipose and liver tissue (Samuel et al. (2001)) (Milanesi et al. (2008)) and in sheep it is expressed in udder Daniel et al. (2004). In mice, 4 types of SCD gene were identified from 4SCD1-SCD and are expressed in a tissue-specific way, i.e. SCD1 is the main gene in adipose tissue and liver and SCD2 is expressed mainly in the brain (Peter et al., 2008).

Many genes have been shown to have effects on lipid metabolism, and SCD desaturates several fatty acids (FA) at the cis-δ 9 position in the lactobacillary glands of ruminants (Kromhout et al., 2002). The SCD gene was significantly expressed in the goat's milky gland, specifically subcutaneous adipose tissue (AT) while reduced expression was observed in adipose tissue around the kidneys, indicating a specific range of expression in adipose tissue, these findings are consistent with a study in sheep, which showed that the SCD gene is highly expressed in the milky gland (Bernard et al., 2005; Salmani et al., 2016).

Statistical analysis

The data were analyzed statistically using the Statistical Analysis System–SAS (2012) to study the effect of genetic manifestations of SCD1 genes on the traits studied according to mathematical models, significant differences between averages were compared using the Duncan (1955) polynomial test in light of the application of the least square means method.

$Y_{ijklm} = \mu + SCD1_i + A_j + S_k + O_l + e_{ijklm}$

Whereas:

Y_{ijklm}: Viewing value.

µ: The general average of the trait.

$SCD1_i$: Impact of multiple genetic manifestations SCD1.

Aj: Age impact.

Sk: The impact of gender.

e_{ijkl} : Random error that is distributed naturally with an average of zero and a variance of $\sigma^2 e$.

Allelic frequency was calculated according to the equation of Falconer and Mackay (1996) for each gene.

$$PA = \frac{2 * \text{No. of Homozygous} + 1 * \text{No. of Heterozygous}}{2 * \text{Total number of samples}}$$

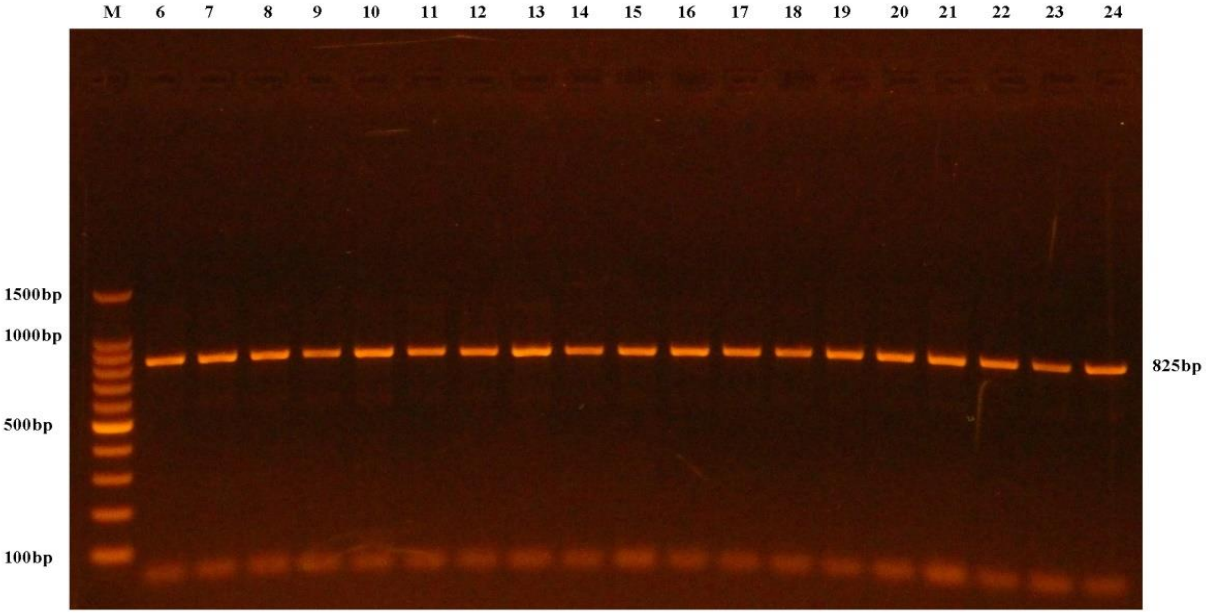
First Allele Repetition: PA

Since: P + q = 1 then the repetition of the second allele is: qB = 1 – PA

3. Results and Discussion

DNA Extraction

DNA was extracted as a first step to extract the SCD gene using the diagnostic kit (Kit) and it was confirmed that the extraction process was successful by migrating all samples electrically on the lacrose gel and as previously shown and the migration product was imaging to confirm the presence of DNA.



SCD1

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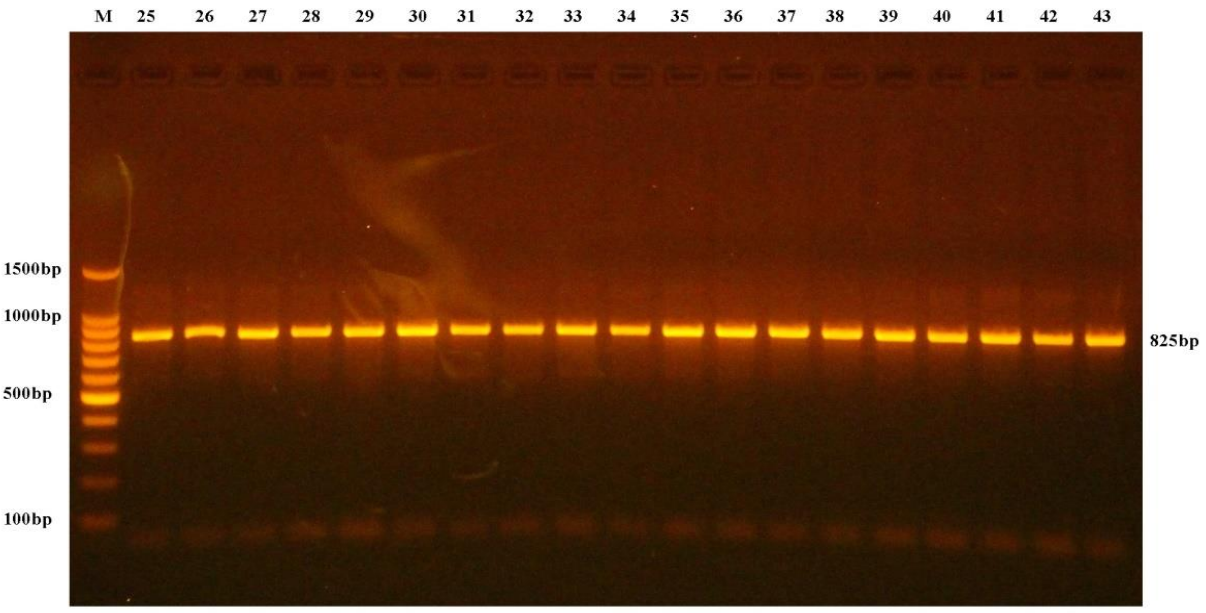

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Studied pieces of the SCD1 gene

The results of the current study using DNA sequencing technology showed the presence of a number of mutations in the studied regions of the SCD1 gene and that the mutations that were detected are all registered with an identification number in the genebank (NCBI) and (ensemble).

The results of the study of the nucleotide sequence of the studied fragment of the SCD1 gene and its relationship with the studied productive traits.

From the results of the electroplating of the products of the polymerase chain reaction, it was found that there were three mutations in the samples of local goats as shown in Table (4-1).

Table (1) Numbers and location of studied area mutations of the SCD1 gene in local goats

Location	Genotype		
	Wild	Hetero	Mutant
G4712A	GG	GA	AA
C4874T	CC	CT	TT
A4933G	AA	AG	GG

Percentages, number and nocturnal frequency of mutation G4712A: 1

It is clear from Table (2) that the proportions of the genetic structures in the mutation (G4712A), namely wild, hybrid and mutant in local goats were (GG: 40.00, GA: 43.33 and AA: 16.67) respectively, and there are no significant differences between the

proportions of the genotypes and the frequency of allelin in wild local goats G and Mutant A (0.62 and 0.38) sequentially, this may be due to the type of breeding adopted at the station and the sample size or to the adaptation of the wild allele to environmental factors, this finding is similar to those of (Kaplanová et al., 2013), and disagreed with (Inostroza et al., 2012).

Table 2 (Percentages of genotype and nocturnal frequency of mutation G4712A)					
Strain	Genotype	No.	Percentages	Significant 2α	allelic frequency
Local	GG	12	40,00	N.S 3,80	G = 0.62
	GA	13	43,33		A = 0.80
	AA	5	16,67		
	Total	30	100%		
NS :Non-Significant					

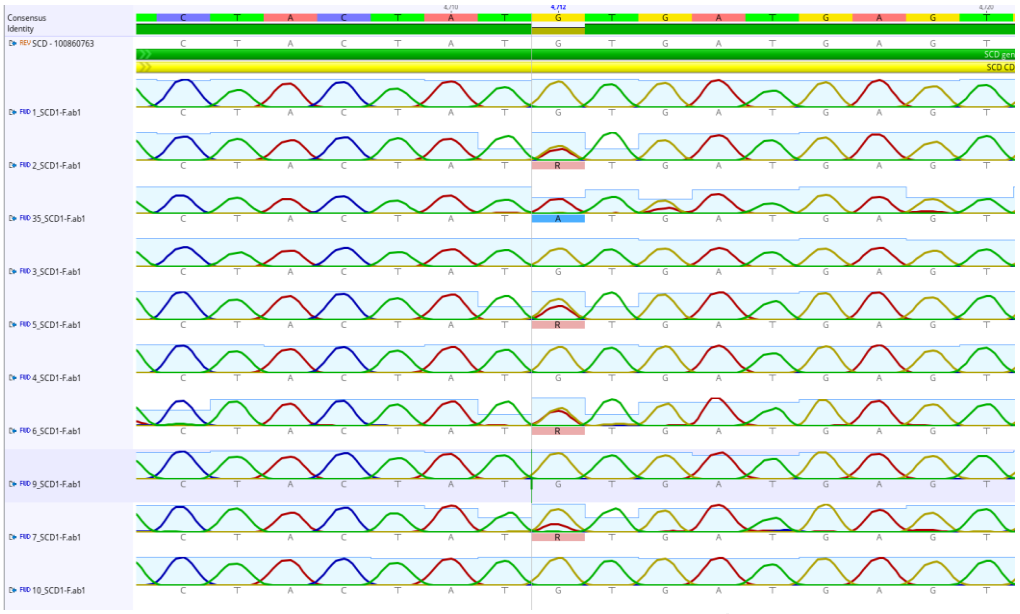

Image (1) G4712A mutation site in the studied piece of the SCD1 gene

The relationship of the G4712A mutation with growth qualities

From Table (3), it is clear that there is no significant relationship between the multifaceted manifestations of the G4712A mutation and the studied growth characteristics represented by the weight of the newborn, weaning weight, the birth weight of the mother and the weaning weight of the mother for local goats, while the live weight outweighed the wild composition GG and hybrid GA on the mutated structure AA, as it was 39.91, 39.76 and 36.20 kg respectively, this may be attributed to the type of breeding adopted at the station and the sample size or to the adaptation of the wild allele to environmental factors, and this result is similar to the results of the study of (Kaplanová et al., 2013).

Strain	Trait	Measuring unit	Genotype			Significant
			GG	GA	AA	
			12	13	5	
Local	Baby weight	Kg	2.85±0.22	2.89±0.17	2.84±0.10	n.s
	Weaning weight	Kg	15.75±0.97	14.30±0.81	14.80±0.58	n.s
	Birth weight	Kg	2.75±0.07	2.92±0.18	2.70±0.12	n.s
	Weaning weight	Kg	20.25±0.62	19.92±0.65	20.80±0.66	n.s
	Live weight	Kg	39.91a± 1.79	39.76a± 1.77	36.20b± 1.88	*
) *P≤0.05، (Non-Significant:N.S						

The relationship of the G4712A mutation with fertility qualities, milk production and components

From Table (4), it is clear that there is no significant relationship between the multifaceted variation of the G4712A mutation and the fertility traits of local goats, as well as milk production and peak production, except for the length of the season, the wild GG genotype outperformed the hybrid GA and mutant AA, reaching 237.08, 217.53 and 200 days respectively. The variation in the results of the studies indicates the existence of interactions between the alleles of the SCD gene and the

occurrence of genetic mutations, as well as the difference in the number of observations according to the genetic manifestations of this gene, increasing the number of samples for different herds and studying more than one piece of the same gene would give more accurate results due to differences in genetic diversity between local breeds as well as differences in management and production systems that led to a significant deterioration in milk production characteristics in all farm animals.

As for the proportions of milk components, there is also no significant relationship except for the percentage of fat, as the wild genetic type GG outweighed the hybrid GA and mutant AA, as it reached 2.69, 1.54 and 1.33%, respectively, and this is consistent with the findings of some studies (Garcia-Fernandez et al. 2010), (Izadi et al. 2016).

Strain	Trait	Measuring unit	Genotype			Significant
			GG	GA	AA	
			12	13	5	
Local	Fertile	Born	1.66±0.22	1.38±0.14	1.60±0.40	n.s
	Milk production	Kg	252.43±42.20	188.40±26.93	173.20±43.60	n.s
	Season Length	Day	237.08a±2.97	217.53ab±5.50	200.00b±19.40	*
	Top Production	Day	35.58±1.93	33.92±1.59	38.00±4.12	n.s
	Protein	%	3.02±0.01	3.05±0.05	3.06±0.05	n.s
	Fat	%	2.69a±0.32	1.54b±0.12	1.33b±0.13	*
	Lactose	%	4.29±0.03	4.33±0.04	4.43±0.03	n.s
	SNF	%	8.17±0.11	8.26±0.12	8.12±0.08	n.s
) *P≤0.05، (Non-Significant:N.S						

Percentages, number and nocturnal frequency of mutation C4874T

It is clear from Table (5) that the proportions of the genotypes in the mutation (C4874T), namely wild, hybrid and mutant in local goats were (CC: 73.33, CT: 23.33 and TT: 3.3) respectively, there were significant differences between the proportions of the genotypes and the frequency of allelin in wild local goats C and mutant T (0.85 and 0.15) was sequential.

Strain	Genotype	No.	Percentages	Significant 2χ	Allelic frequency
Local	CC	22	73.33	**37.93	C = 0.85 T = 0.15
	CT	7	23.33		
	TT	12	3.3		
	Total	30	100%		
(**P≤0.01)					

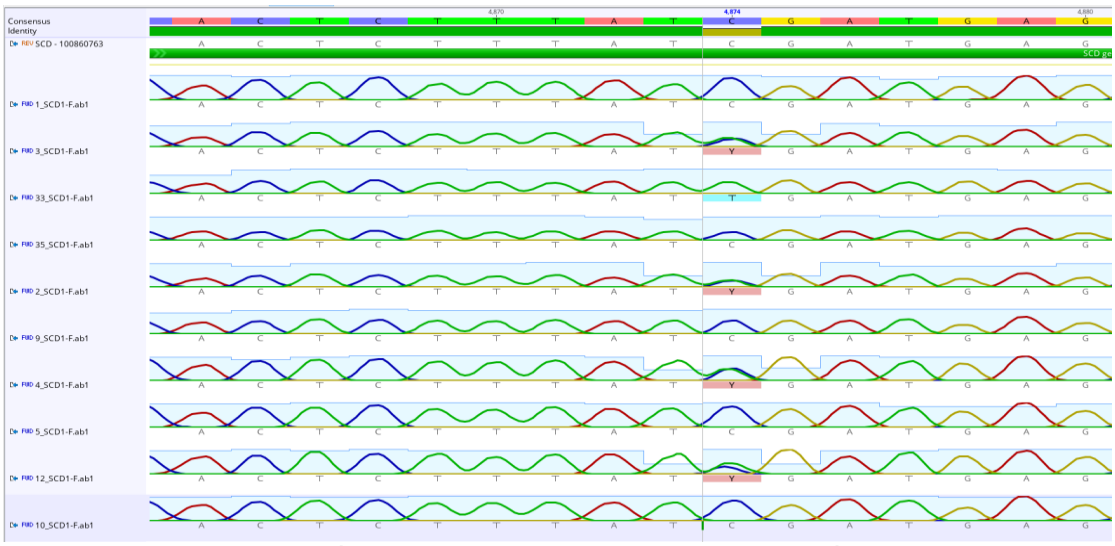

Image (2) Location of the C4874T mutation in the studied piece of the SCD1 gene

The relationship of the C4874T mutation with growth qualities

From Table (6), it is clear that there is no significant relationship between the multiplicity of manifestations of the C4874T mutation and the studied growth qualities represented in the weight of weaning and the birth weight of the mother and the weight of weaning of the mother for local goats except for the weight of the newborn, where the mutant composition TT exceeded the hybrid CT and wild composition CC, as it reached 4.50, 3.07 and 2.72 kg, respectively, and there is a significant difference for live weight, where the mutant composition TT outperformed the wild CC and CT hybrid compositions, as it reached 46.00, 40.09 and 35.57 kg, respectively.

Table (6): Relationship of mutation C4874T with growth traits						
Strain	Trait	Measuring unit	Genotype			Significant
			CC	CT	TT	
			22	7	1	
Local	Baby weight	Kg	2.72b±0.12	3.07b±0.17	4.50a±0.10	*
	Weaning weight	Kg	14.72±0.61	15.14±1.61	19.00±0.21	n.s
	Birth weight	Kg	2.86±0.11	2.64±0.14	3.00±0.12	n.s
	Weaning weight	Kg	19.95±0.50	20.85±0.40	21.00±0.66	n.s
	Live weight	Kg	40.09b±1.33	35.57b±1.21	46.00a±1.45	*
) *P≤0.05، (Non-Significant:N.S						

The relationship of mutation C4874T with fertility qualities and milk production and its components

From Table (7) it is clear that there is no significant relationship between the polymorphism of the C4874T mutation and the fertility traits of local goat individuals, as well as milk production, season length, peak production and milk components, except for SNF, where the wild CC genotype outperformed the CT and mutant hybrid structure TT, reaching 268., 8.12 and 7.45%, respectively.

Table (7): The relationship of mutation C4874T with fertility traits and milk production and its components						
Strain	Trait	Measuring unit	Genotype			Significant
			CC	CT	TT	
			22	7	1	
Local	Fertile	Born	1.68±0.15	1.14±0.14	1.00±0.21	n.s
	Milk production	Kg	216.59±27.80	174.47±27.55	358.05 ±41.60	n.s
	Season Length	Day	226.36±4.17	207.28±14.19	242.00±17.30	n.s
	Top Production	Day	35.22±1.37	35.85±3.10	32.00±2.12	n.s
	Protein	%	3.03±0.03	3.07±0.04	3.04±0.04	n.s
	Fat	%	1.96±0.17	1.93±0.58	2.24±0.17	n.s
	Lactose	%	4.12±0.03	4.34±0.05	4.33±0.02	n.s
	SNF	%	7.45b±0.06	8.12a±0.05	8.26a±0.08	*
) *P≤0.05، (Non-Significant:N.S						

Percentages, number and night frequency of the A4933G mutation

Table 8 shows the proportions of the genotypes in the mutation (A4893G) namely wild, hybrid and mutant in local goats were (AA: 33.34, AG: 43.33 and GG: 23.33) respectively, and there were no significant differences between the proportions of the genotypes of local goats.

Table(8) Percentages of genetic makeup and nocturnal frequency of mutation A4933G					
Strain	Genotype	No.	Percentages	Significant 2χ	Allelic frequency
Local	AA	10	33.34	N.S 1.133	A = 0.55 G = 0.45
	AG	13	43.33		
	GG	7	23.33		
	Total	30	100%		
Non-Significant:N.S					

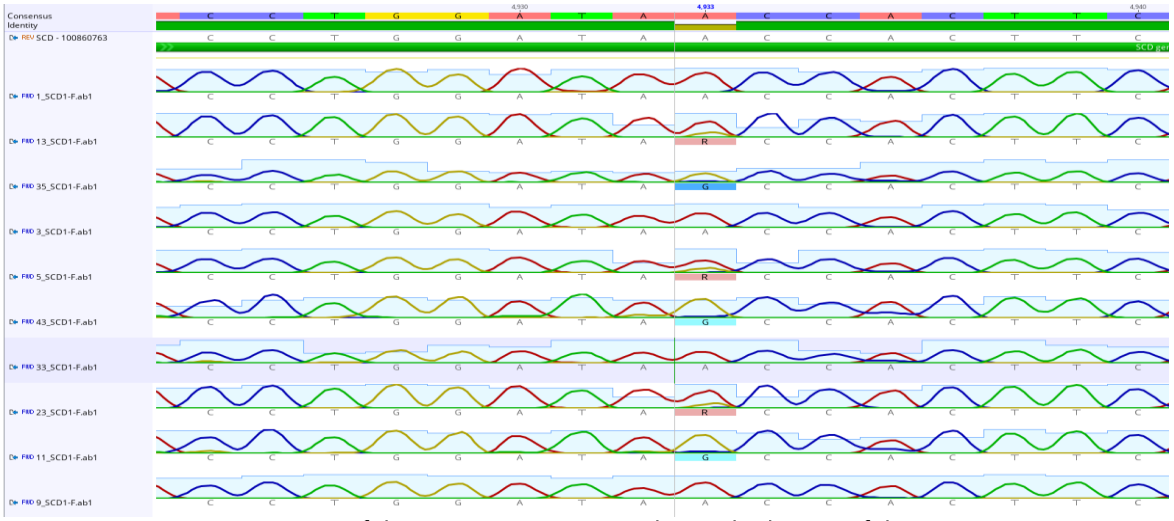

Image (3) Location of the A4933G mutation in the studied piece of the SCD1 gene

The relationship of the A4933G mutation with growth qualities

From Table (9), it is clear that there is no significant relationship between the multiple manifestations of the A4933G mutation and the studied growth characteristics represented by the weight of the newborn, weaning weight, birth weight of the mother and the weight of weaning of the mother, except for the live weight of the local goats, where the hybrid genetic type AG outweighed the wild structure AA and the GG mutant, where it was 40.53, 39.80 and 36.00 kg, respectively.

Table 9 Relationship of mutation A4933G with growth traits

Strain	Trait	Measuring unit	Genotype			Significant
			AA	AG	GG	
			10	13	7	
Local	Baby weight	Kg	3.02±0.23	2.70±0.16	2.95±0.19	n.s
	Weaning weight	Kg	15.50±1.12	15.00±0.83	14.14±0.67	n.s
	Birth weight	Kg	2.75±0.08	2.96±0.17	2.64±0.14	n.s
	Weaning weight	Kg	20.30±0.70	19.46±0.53	21.42±0.75	n.s
	Live weight	Kg	39.80a±2.30	40.53a±1.59	36.00b±1.30	*
) *P≤0.05، (Non-Significant:N.S						

The relationship of the A4933G mutation with fertility qualities, milk production and components

From Table (10), it is clear that there is no significant relationship between the multiplicity of manifestations of the A4933G mutation and the fertility traits of local goats, as well as milk production, peak production, season length and milk components except for the percentage of fat and lactose, where the wild genetic type AA exceeded the hybrid structure AG and the GG by the percentage of fat, reaching 692., 1.66 and 1.37 % respectively, which is consistent with the findings of some studies (Garcia-Fernandez et al., 2010 and Izadi et al., 2016). Lactose outperformed the GG mutant genotype over the AG and wild AA hybrid genotype at 4.43, 4.30 and 4.29%, respectively, there were significant differences (P≤0.05) for the effect of the SCD gene in the proportion of solids according to different genetic structures, the percentage of fat is one of the most important structural characteristics of milk that determine the quality of milk and its price and the type of product from which it is made, and then the adoption of gene expression in improving this trait seems feasible through the results of this study, just as the presence of some alleles in an animal's genome can negatively affect the percentage of milk fat, so too do alleles increase the percentage and also affect milk composition and udder health (Abdel Hameed et al., 2006,), there is also an inverse relationship between the amount of milk produced and the percentage of fat in milk.

Table (10): The relationship of mutation A4933G with fertility traits and milk production and its components

Strain	Trait	Measuring unit	Genotype			Significant
			AA	AG	GG	
			10	13	7	
Local	Fertile	Born	1.50±0.22	1.61±0.18	1.42±0.29	n.s
	Milk production	Kg	270.87±48.94	192.20±26.21	162.44±31.23	n.s
	Season Length	Day	230.87±5.59	225.07±5.71	205.71±13.95	n.s
	Top Production	Day	36.50±2.23	33.30±1.46	37.14±3.18	n.s
	Protein	%	3.02±0.01	3.02±0.04	3.11±0.06	n.s
	Fat	%	2.69a±0.32	1.66b±0.15	1.37b±0.15	*
	Lactose	%	4.29b±0.03	4.30b±0.04	4.43a±0.02	*
	SNF	%	8.23±0.13	8.20±0.12	8.15±0.06	n.s
) *P≤0.05، (Non-Significant:N.S						

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