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Correlation between Some of Pitx2 Snps with Milk Production and Contents in Goat

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Annotation: The study was conducted at the ruminant research station of the Agricultural Research Department / Ministry of Agriculture for the period from 1-1 2022 to 4-3 2022 for the purpose of determining the single nucleolide polymnorphisms (SNPs) of the Paired like homoedomain transcription factor 2 gene and milk production. 52 A goat under the same rearing conditions (16 local goats and 36 Shami goats). The results of the current study showed the emergence of four mutations in the operating region of the gene, according to the DNA sequencing method. The size of the studied plot 966 base pairs resulted in the mutations G1148A and G1089A, which resulted in two genotypes: wild GG and hybrid GA with the absence of the AA mutant, while three different genotypes emerged at the mutation C1141A and they were wild CC, CT hybrid, TT mutant, and the mutation G1003A emerged from them. Structures, wild, hybrid and mutant GA, GG, AA. The difference of the strain had a significant effect (05.P < 0) on the length of the milk season, the rate of daily milk production and the percentage of protein in the milk. The total milk production rate was not affected in addition to the percentages of fat, lactose and solids in the milk. The percentage of protein in milk differed according to the genotypes within the G1148A mutation. The G1089A mutation caused a variance in

the length of the milk season, the daily and total milk production rate, and the percentage of fat in that the wild formula GG was superior to the values of these traits than the GA compound. The G1003A mutation was significant in the length of the milk season with the ratios of both protein and fat in milk (05.P < 0). The Shami goats outperform the local ones in the length of the milk season, the total milk production rate and the protein content in the milk, which reached 65.198 days, 78.317 kg and 77.2% for the local one. The GG wild model was higher in the protein content in the milk 95.2%, while it reached 71.2% for the GA hybrid. Within the G1148A mutation. About the G1089A mutation, individuals carrying the wild formula GG recorded the highest measurements of the length of the milk season and the average daily and total milk production, which were 89.194 days, 37.350 g and 28.68 kg, in addition to the percentage of fat in milk 58.2%. The G1003A mutation produced the highest longest seasonal milk, which is 50,221 days for the mutant AA. As for the milk content of protein and fat, it was 12.3% and 61.3% higher than that of the wild and hybrid formulations.

Introduction

Genetic improvement in animal production depends on knowledge of the genetic value of the animal and its transmission from one generation to another. In addition to the scarring, which is determined according to the value of the equivalent of multiple traits, as its value increases, the speed of genetic improvement increases (Khalil and colleagues, 2007) because the selection process includes selecting the parents who excel in them. The values of the specific trait. The balance between the increase in the speed of genetic improvement and the resulting increase in kinship between members of the herd, which negatively affects the economic characteristics. The selection process is based mainly on the availability of information and therefore the accuracy of the selection of parents. This leads to the average genetic values in the generation of offspring being equal to the generation of the selected fathers to ensure obtaining lambs or calves with high productivity traits, whether meat or milk. The amount of genetic improvement across generations depends on the breeder's experience in identifying ruminants (Khalil and colleagues, 2009). The interest in animal production is escalating, which stimulates interest in all scientific aspects that specialize in breeding systems, nutrition and health care. The interest in raising livestock is emerging not because it is one of the important components of animal production, but because it has a large contribution to agricultural income and also provides the population's need for animal products, which included meat, milk and its products, and benefit from Of milk, which is one of the most important products in the manufacture of several products, including cheese, coffee and butter (Alraee et al., 2004).

Goats can benefit from various feeds, so their breeding depends on the use of the breeder. Despite the existence of other breeding systems, they are generally intensively bred all over the world, especially in Asia and Africa (Steinfeld and colleagues, 2013), especially breeds with

high production. This reduces costs, increases its quality and increases the period of vegetation growth due to its residues (Lopes and colleagues, 2012) and it has been proven that there is a statistically significant relationship between environmental factors and gene expression levels through studies conducted on many animal species (Bernard and colleagues, 2009, Richards and colleagues, 2003). In a study on alpine goats, oillier and colleagues (2007) found that expression levels of 161 genes in Udder tissues affect milk production and quality standards. Most of the economic traits in livestock are affected by a large number of genes in addition to their impact on the environment. Among these traits are the speed of growth, milk production, etc., and here it turns out that increasing the proportion of good genes improves the genetic value of animals and increases their production (Al Jamali and colleagues, 2018). Given that goats are an economical, multi-purpose animal, this is due to many of the traits they are characterized by, depending on the many genes carried by the animals.

Milk production and its features are quantitative traits that are controlled by many genes that have a precise effect. Therefore, the molecular markers of DNA are important in improving these traits. The genes PROP and Pou1f1 affect the expression level of GH and prl associated with milk traits, and the genetic variants of the Pou1f1 genes (Lan et al. PROP (Lan et al., 2013a), lhx3 (Li et al., 2008), lhx4 (Liu et al., 2011) and pitx2 (Lan et al., 2013b) are significantly related to these traits. However, information about genetic variation of the pitx2 gene and its effects on milk traits in the Goats are rare because the pitx2 gene has an indirect effect through the B-catenin Wnt pathway and the Pou1f1 pathway, and the pitx2 protein acts as a transcriptional regulator involved in prolactin activity. Genotypes A and C resulting in synergistic activation of the PLOD and DIX2 operating region (Fahim et al., 2017). Given the importance of this gene in cattle and its impact on their productive performance, this study was conducted to show the effect of the gene on milk production and its components in goats, with the identification of genetic structures and mutations within the gene in the operating area. There are many ways to improve milk production providing suiable environmental conditions of the rumen and exploitation of nutrients compounds and metabolism in addition to health care of the somatic cells of the udder.Animals need good feed to produce milk.Notes after birth, and there is a growing need for animal food to produce milk and this may render the animal in the case of the balance of negative especially if the production is high (Economides et al.,1986),may be exposed females after giving birth to stress due of milk, especially those with highly productive result of the deterioration of the immune status and low concentration antioxidants in the body, leading to increased opportunities for exposure to disease, particularly mastitis (Moeini et al.,2009، Merkhan et al.,2019).

Materials and Methods

The study was carried out at the ruminant research station of the Agricultural Research Department \ Ministry of Agriculture, on two samples of goats, one of them consisted of 21 local goats and the other consisted of 36 Shami goats aged (1–5) years. This is for the field part, while partial tests were conducted in laboratories Scientific progress specialized in partial genetics and technologies with the aim of determining the genotypes of the PITX2 gene and its relationship to some economic traits in goats. Milk samples were also analyzed in the Abu Ghraib factories for dairy products of the General Company for Food Industries.

Animals are raised in semi-open pens (35% roofed, 65% open) designated for sheltering. The herd is managed according to a program that includes feeding, preparation for the shedding season, preparation for pregnancy and childbirth, as well as health and veterinary care.

The quantity and quality of fodder varies with different seasons and according to its availability. Coarse fodder, such as crushed hay, green fodder, or jet hay, is provided. Concentrated fodder is provided at an amount of 500 g/animal per day. This quantity increases before and during the reproductive season for goats and goats, and the amount of concentrated fodder allocated to each goat is increased in a season. Childbirth (lactation and milk production) The composition of the

feed varies according to the productive state of the animals, with the provision of mineral salts briquettes. As for feeding the newborns, they are left with their mothers to breastfeed, as they start at the age of two weeks by eating small amounts of feed up to 55 g/day of concentrated feed, in addition to the progress of green fodder and powdered jet leaves As for camels and weaned females up to one year of age, they are given concentrated feed with a percentage of 3% of body weight and free roughage, and the newborns are weaned at the age of about 12 days.

Milk production and analysis of milk samples

The animals were milked once a day, as the newborns were isolated from the mothers in the evening and milked in the morning, and then the newborns were released to the mothers. Milk samples were taken from each animal every two weeks and the milk samples were analyzed to find out the percentages of milk components (fat, solid non-fat components, protein, and lactose). Using the device (LM2-Maste classic), which works with a new type of signals that pass through the measurement sensor, then calculate the length of the milk season, as well as the period during which the animal reaches the peak of production. The total milk production was also calculated from the following equation:

Total milk production (kg) = average daily milk production x number of days of milking.

DNA Extract

DNA was extracted from blood samples drawn using the kit (kit) and according to the applicable contexts from the company that supplied ReliaPrep Blood Gdna Miniprep system, Promega

DNA extraction method

1. Blood samples were mixed completely for 15 minutes using a Roll Mixer at room temperature.
2. 1.5 mm centrifuge tubes were used and 20 µl of Proteinase K (PK) solution was distributed to the tubes, then 200 µl of blood sample was added to it and then the mixture was mixed manually.
3. Add 200 µl of Cell Lysis Buffer (CLD) solution to the tubes and then mix them using a vortex device for 10 seconds.
4. Put the tubes containing the mixture in a water bath at 56 degrees Celsius for 30 minutes.
5. Meanwhile, the ReliaPrep Binding Column placed filter tubes are prepared and placed in collection tubes intended for the centrifuge.
6. After removing the tubes from the water bath, add 250 µl of Binding Buffer solution to each tube and mix using a vortex device for 10 seconds.
7. Transfer all the contents of tubes to the ReliaPrep Binding Column tubes and place them in new collection tubes.
8. After the centrifugation process is completed, the collection tubes containing the clear solution are destroyed and the remaining residue is kept in the ReliaPrep Binding Column tubes and placed in new collection tubes.
9. After that, 500 microliters of Column Wash Solution (CWD) are added to each tube and the tubes are centrifuged for 3 minutes at full speed, then the sludge is removed, observing this step three times.
10. After that, the washing step is completed. The tubes containing the precipitate are placed in 1.5 ml micro centrifuge tubes, 100 microliters of Nuclease Free Water are added to them and then left for 5 minutes.
11. The tubes are centrifuged at 5000 cycles for 5 min.

12. Then the tubes containing the filter are discarded and the clear solution containing the genetic material is kept.

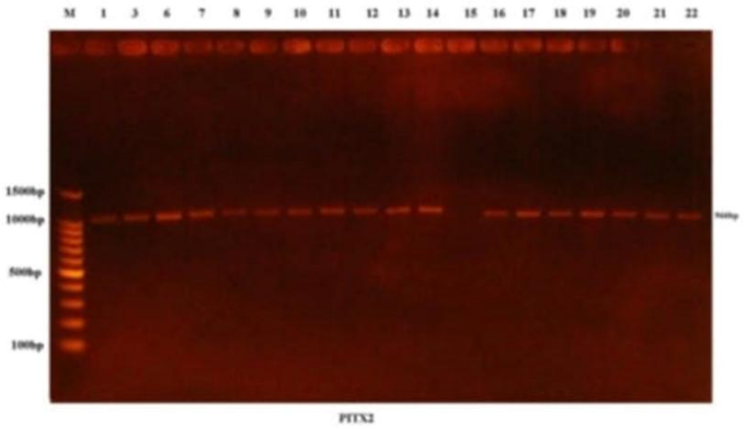
DNA Genetic Material Loading:

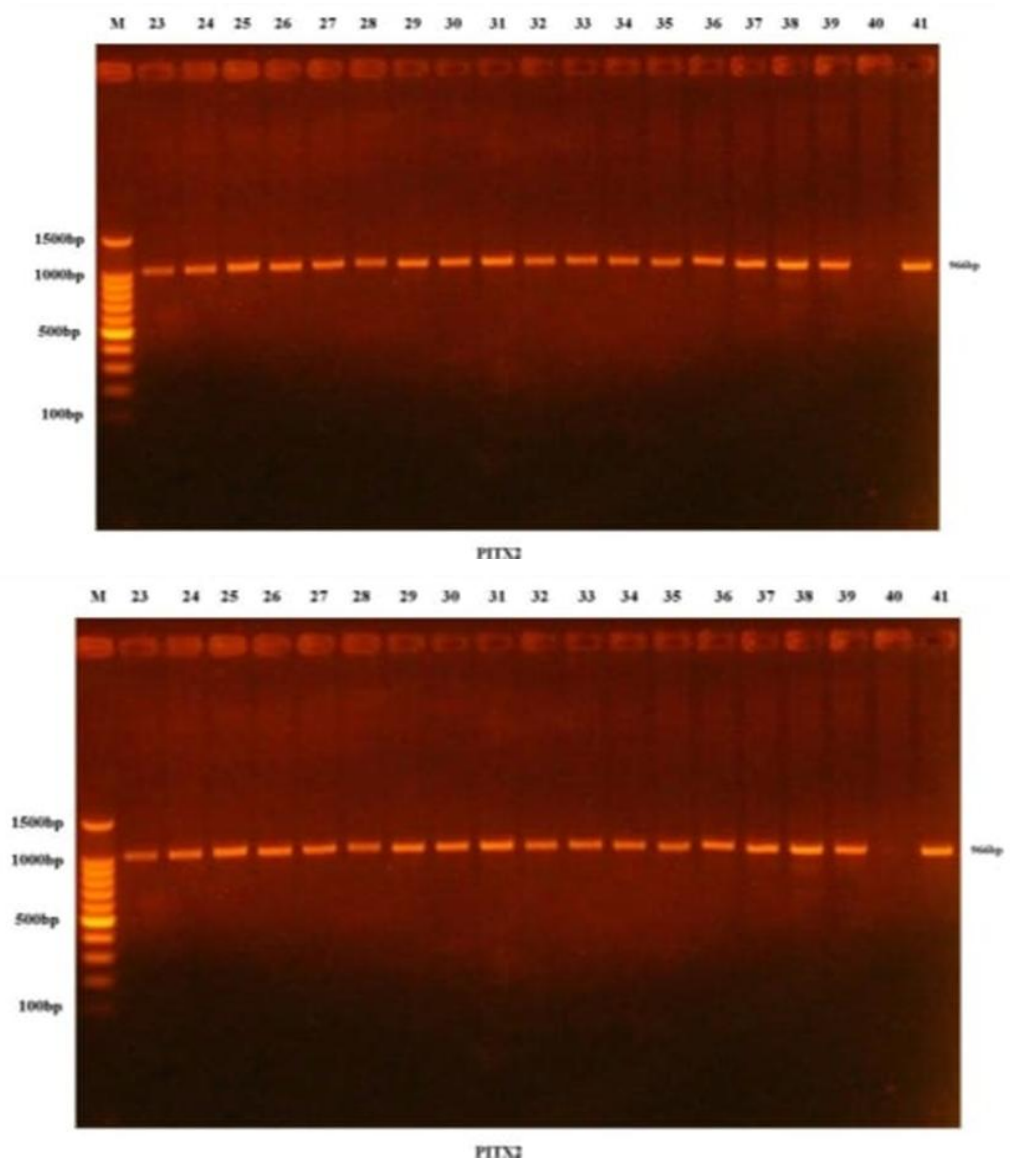
For PCR product 5 µl was loaded directly into the well. The electrical power was run at 100 V and 50 mA for 60 minutes. The DNA moves from the cathode to the trailing anode electrodes. Ethidium bromide-stained samples were photographed in the gel using a gel imaging system.

DNA Electrophoresis:

It is one of the methods used to verify the presence of DNA extracted from blood samples. Electrophoresis is carried out in several steps:

1. Dissolve 1 g of 10% agarose powder in 100 ml of TAE1X in a glass beaker.
2. Put the solution in a Microwave electric oven for 1.5 minutes in order to melt all the minutes of the agarose by heating, making sure that the agarose is gone.
3. Leave the solution for several minutes to cool so that it is warm and at a temperature of 50-60 degrees Celsius.
4. Then add 1 microliter of Ethldium Bromide stain (10 mg/mm) and mix it well with the gel.
5. The casting mold is prepared as the two edges of the casting plate are closed by a special holder for the plate and the comb is placed to make drilling (wells) in the gel, and then pour the gel quietly to prevent the occurrence of air bubbles, taking into account that the surface is flat.
6. Leave the gel mold to harden for 10-30 minutes depending on room temperature.
7. Then carefully remove the comb and then place the gel template in an electrophoresis buffer TAE1X tank filled with Electrophoresis Buffer TAE1X until the gel is completely immersed until the solution reaches 2 mm above the surface of the hardened gel.
8. Several drops of Bromophenol Blue Dye were placed over a clean smooth paper strip from the table at the rate of 2 microliters for each drop for several samples and then 6 microliters of DNA were placed on top of each drop and then mixed with it with the pointed tip of the straw. Then the mixture was withdrawn by the absorbent device (Micropipette) placed in the prepared gel pits after removing the comb from the gel.
9. Carefully load the DNA extracted from the samples into the holes made in the gel mold.
10. Then the samples are transferred by running the Electrophoresis Gel on an electrical power of 100 mA for 85 minutes, and then the gel layer is transferred after the specified time to the UV Light Transillminator for the purpose of viewing the DNA bundles and imaging these bundles with a camera. Especially as the bands appear colored with ethidium bromide dye in a bright orange color, which indicates the presence of DNA.





Results of the amplification of PITX2 gene were fractionated on 1.5%agarose gel electrophoresis with Eth.Br.M:100bp ladder marker.

Quantitation of DNA

The concentration of the extracted DNA was measured using (Quantus Fluorometer) by adding 1 microliter of the extracted DNA to 200 microliters of Quanty flour Dye diluent and then mixing them well, then the mixture was left for 5 minutes at room temperature and then the concentration of DNA was measured.

As for the measurement of the purity of the DNA, it was done using the Nanodrop device, as the use of this device made this process easy and fast, as well as revealing to us the percentage of error that might exist in the sample, as the standard readings of the DNA are DNA = 8.1 As for the readings that differ from these The percentages are an indication of the presence of contamination in the sample, meaning that the sample still contains protein or some other material. The reading was carried out at a wavelength of 260-280 nm.

How the device works:

1. Select the device icon (Nanodrope) on the desktop.
2. Choose Nucleic acid from the list.
3. Selection of the type of DNA methylation.

4. Choosing the unit of measurement for nucleic acid, which is ul ng
5. Choosing the appropriate wavelength for the solution to be examined (the 280-260 wavelength is appropriate for measuring and estimating DNA).
6. Choose the (Add to the report) icon to add all the measurements for all samples and save them until you refer to them when needed.
7. Zeroing the device using the solvent solution of the nucleic acid (Blank), and this solution must be a good solvent for the nucleic acid, in addition to the Blank used to yellow the device, it must be at the pH level and the ionic degree of the solution used to solve the nucleic acid.
8. Put 1-2 microliters of the solvent solution on the lens of the device, then lower the device arm and press the word (Blank).
9. Clean the lens with a special cleaning paper, then put the sample and press the command (Measure) for the device to start measuring.

Statistical Analysis

The data were statistically analyzed using the program Statistical Analysis System–SAS (2012) to study the effect of the genetic phenotypes of the PITX2 gene on the studied traits according to the mathematical model below, and the significant differences between the means were compared using Duncan (1955) polynomial test by applying the method of least squares averages (Least). square means).

Mathematical model: the relationship of the genetic phenotypes of the PITX2 gene to the traits studied:

$Yijkm = \mu + Gi + Aj + Sk + Tl + eijklm$

Yijkm: watch value m.

μ: the general average of an adjective.

Gi: the effect of genetic phenotypes of the PITX2 gene and for each SNP, for both Shami and local goats.

Aj: the effect of age (2-5 years).

Sk: influence of gender (male, female).

Tl: influence of the type of birth (single, twin, triple).

eijk: the naturally distributed random error with a mean of zero and a variance of σ2e.

Comparison between local and Shami goats in the characteristics studied on mothers and newborns

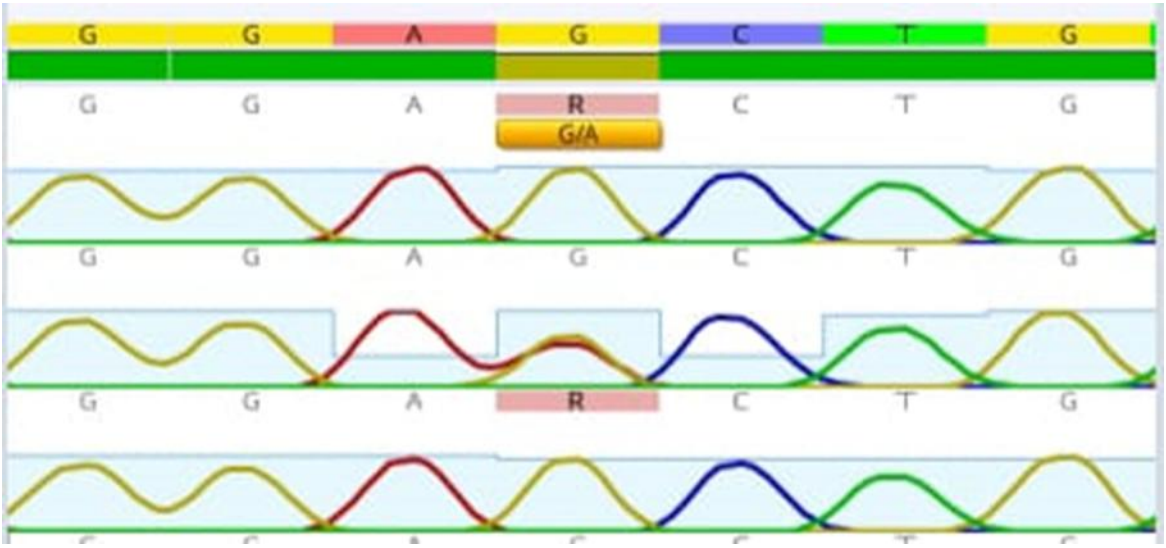
It is clear from Table (1) that there were significant differences (05,P <0) in the length of the milk season, which amounted to 75,179 days and 65,198 days, and the daily milk production rate was 78,317 g and 28,354 g, in addition to the percentage of protein in milk 77.2% and 59.2% Respectively, while the strain had no effect on the rate of total milk production, as the quantity of Shami goats outperformed the local one, reaching 12.57 kg and 37.70 kg, respectively, in addition to the fact that the percentage of fat, lactose and solids in milk was not affected by the different breed.

Table (1): Comparison between local and Shami goats in the characteristics studied on mothersand newborns

Adjectives	mean ± standard error		LSD
	Domestic Breed)Number = 16(	Shami dynasty (Number = 36)	
Length of the milk season (day)	8.14± 179.75b	7.53± 198.65a	*
Daily milk production rate (gm)	35.98± 317.78b	19.46 354.28a	*
Total milk production rate (kg)	292.88± 57.96	146.53± 70.72	NS
Milk protein percentage(%)	0.08± 2.77b	0.02± 2.99a	*
Milk fat percentage (%)	0.19± 2.37	0.15± 2.59	NS
The percentage of lactose in milk(%)	0.05± 4.43	0.06± 4.33	NS
Milk Solids Percentage (%)	0.06± 8.14	0.04± 8.03	NS
insignificant* (P <0.05)، NS The averages with different letters within the same column differ significantly between each other			

The relationship of genotypes in the PITX2 gene with the mutation G1148A in the traits studied on mothers and newborns in goats.

It was found that the structures belonging to the G1148A mutation had a significant effect on the protein content in milk (05 (P < 0), which reached 95.2% and 71.2% for the GG and GA genotypes.



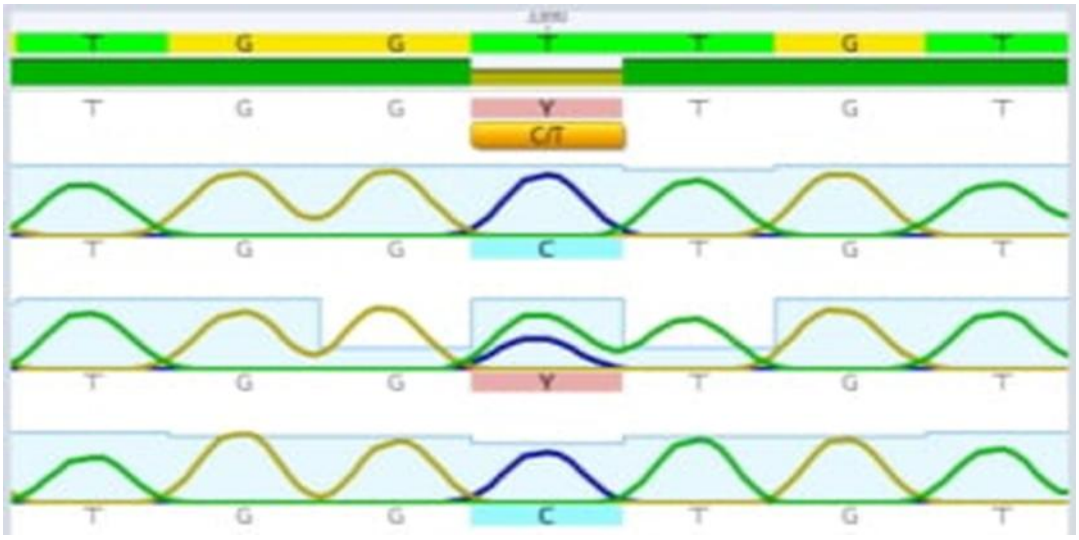
Analysis of G1148A SNP of PITX2 gene using Sanger sequencing .Single G peak indicative of a G homozygous allele.Presence of the G and A peak indicative of G/A heterozygous allele

Table (2): Effect of PITX2 genotypes within the G1148A mutation on the studied traits in goats.

Adjectives	mean ± standard error		LSD
	GG	GA	
Length of the milk season (day)	6.02± 194.74	22.67± 174.20	NS
Daily milk production rate (gm)	18.34± 344.44	58.92± 333.33	NS
Total milk production rate (kg)	110.41± 67.25	13.72± 58.08	NS
Milk protein percentage (%)	0.03± 2.95b	0.19± 2.71a	*
Milk fat percentage(%)	0.13± 2.50	0.25± 2.76	NS
The percentage of lactose in milk(%)	0.05± 4.36	0.07± 4.35	NS
Milk Solids Percentage (%)	0.04± 8.08	0.12± 7.92	NS
insignificant* (P <0.05)، NS The averages with different letters within the same column differ significantly between each other			

Relationship of PITX2 genotypes with C1141T mutation in the studied traits on mothers and newborns in goats.

Milk traits did not differ according to the genotypes (Table 3).



Analysis of C1141T SNP of PITX2 gene using Sanger sequencing .Single C peak indicative of a C homozygous allele.Presence of the C and T peak indicative of C/T heterozygous allele

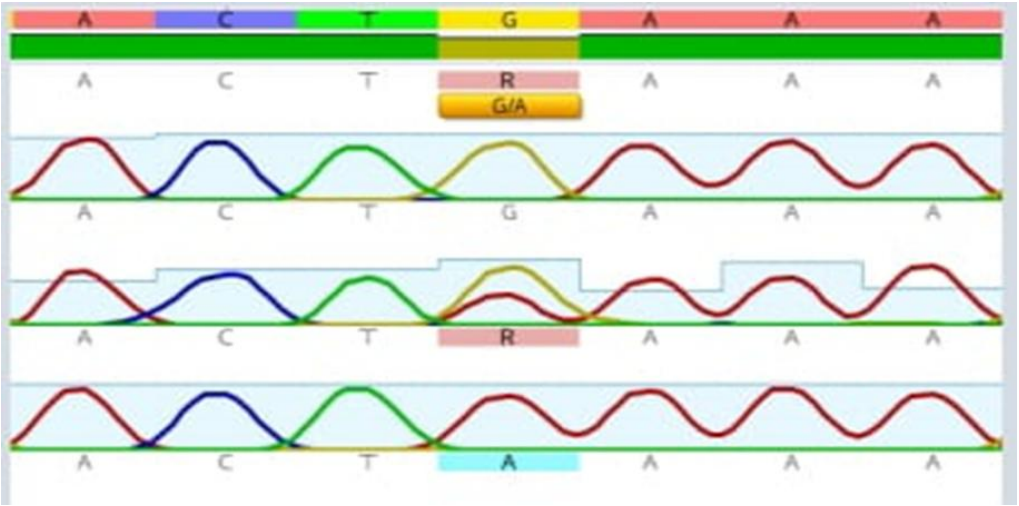
Table (3): Effect of PITX2 genotypes within the C1141T mutation on the studied traits in goats.

Adjectives	mean ± standard error			LSD
	CC	CT	TT	
Length of the milkseason(day)	15.62± 196.43	9.32± 188.67	8.77± 194.54	NS
Daily milk production rate	50.89± 373.81	26.50± 337.96	25.99± 338.66	NS

(gm)				
Total milk production rate (kg)	440.71± 73.50	246.98± 64.91	227.93± 66.92	NS
Milk protein percentage(%)	0.16± 2.83	0.05± 2.88	0.03± 2.98	NS
Milk fat percentage(%)	0.29± 2.85	0.22± 2.34	0.16± 2.57	NS
The percentage of lactose in milk(%)	0.07± 4.36	0.09± 4.37	0.06± 4.34	NS
Milk Solids Percentage(%)	0.17± 7.96	0.05± 8.12	0.04± 8.05	NS
insignificant) *P<0.05 ،(NS The averages with different letters within the same column differ significantly between each other				

Effect of PITX2 genotypes with mutation G1089A on the traits studied in goats

It was obtained from Table (4) that the different models affected the milk characteristics (P<0.05). The individuals carrying the GG strain within the G1089A mutation were superior in the length of the milk season, the daily milk production rate and the total milk production rate, reaching 89,194 days, 37,350 gm and 28.68 kg, while these outperformed The traits in the mixed individuals were 26,167 days, 33,283 g and 37.47 kg (P< 0.05), respectively. The percentage of fat in milk increased to 58.2% for the GG model and decreased to 94.1% for the GA hybrid (P < 0.5).



Analysis of G1089A SNP of PITX2 gene using Sanger sequencing .Single G peak indicative of a G homozygous allele.Presence of the G and A peak indicative of G/A heterozygous allele

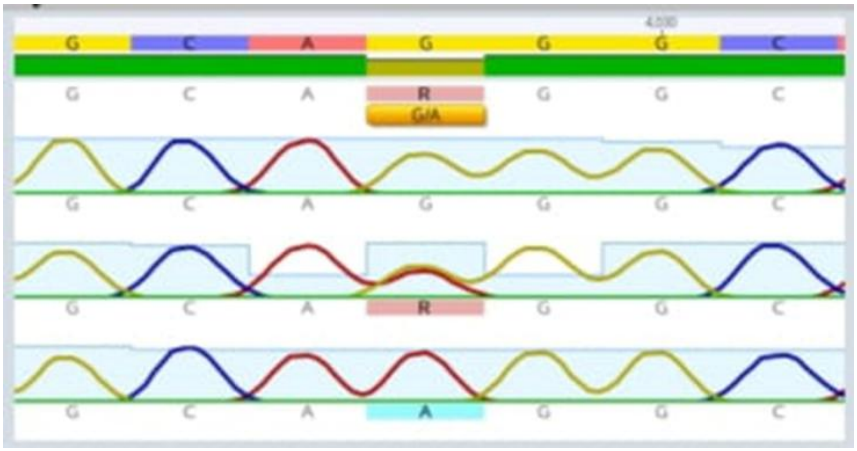
Table (4): Effect of the genetic structures of the PITX2 gene within the G1089A mutation on the traits studied in goats.

Adjectives	mean ± standard error		LSD
	GG	GA	
Length of the milk season (day)	6.34± 194.89b	12.76± 167.20a	*
Daily milk production rate (gm)	18.98± 350.37b	41.49± 283.33a	*
Total milk production rate (kg)	68.61b120.33±	47.78a529.41±	*
Milk protein percentage(%)	0.03± 2.93	0.08± 2.86	NS
Milk fat percentage(%)	0.12± 2.58b	0.34± 1.94a	*

The percentage of lactose in milk(%)	0.05± 4.35	0.05± 4.43	NS
Milk Solids Percentage(%)	0.04± 8.06	0.06± 8.03	NS
insignificant) *P <0.05 ،(NS The averages with different letters within the same column differ significantly between each other			

Effect of PITX2 genotypes with mutation G1003A on the traits studied in goats

On the other hand, the longest milk season was 50,221 days for the AA mutant, and it decreased to 43,192 days and 83,188 days for the wild and hybrid, in addition, the protein and fat percentage in the milk increased to 12.3% and 61.3% for the AA composition. The lowest amounted to 78.2% and 45.2% for the hybrid and 96.2% and 49.2% for the wild GG (P<0.5) in the same context. The daily and total milk production rate and the percentage of lactose did not differ by the diversity of genotypes (Table 5)



Analysis of G1003A SNP of PITX2 gene using Sanger sequencing .Single G peak indicative of a G homozygous allele.Presence of the G and A peak indicative of G/A heterozygous allele

Table (5): Effect of the genotypes of the PITX2 gene within the G1003A mutation on the traits studied in goats.

Adjectives	mean ± standard error			LSD
	GG	GA	AA	
Length of the milk season (day)	7.25± 192.43	10.77± 188.83	1.50± 221.50	*
Daily milk production rate (gm)	18.51± 337.96	343.05 44.445±	108.33± 441.67	NS
Total milk production rate (kg)	134.20± 65.64	478.67± 65.13	126.50± 98.91	NS
Milk protein percentage(%)	0.03± 2.96ab	0.10± 2.78b	0.15± 3.12a	*
Milk fat percentage (%)	0.14± 2.49b	0.25± 2.45b	0.52± 3.61a	*
The percentage of lactose in milk(%)	0.06± 4.32	0.05± 4.43	0.13± 4.55	NS
Milk Solids Percentage(%)	0.04± 8.04	0.09± 8.08	0.17± 8.37	NS
insignificant* (P <0.05)، NS The averages with different letters within the same column differ significantly between each other				

We conclude from the research that the Shami goats outperform the local in some milk characteristics, which included the length of the milk season, the rate of daily milk production and the protein content in the milk, which reached 65.198 days, 28,354 g and 99.2%, respectively. Absence of the AA mutated structure for both G1148A and G1089A mutants in the local and Shami goat breeds. The individuals carrying the GG synthesis within the G1148A mutation had higher milk production than the hybrid individuals, despite the lack of significant effect of the genotype on these traits. The TT model of the C1141T mutation outperformed most of the studied traits except for the percentage of both lactose and solids in the milk, which increased for the hybrid composition, and the length of the season of milk and the rate of daily milk production in addition to the percentage of fat for the individuals with the CC composition was higher. The G1089A mutation for the wild GG model showed the highest length of milk season, daily and total milk production rate, as well as a higher percentage of protein, fat and solids in milk. The AA variant of the G1003A mutation showed an increase in the length of the milk season, the rate of daily milk production, the proportion of protein, fat, lactose and solids. We recommend studying different other regions of the gene to identify the variations in them and their relationship to the performance of goats. Selection of GG-carrying individuals that are distinguished in most of the studied traits that include milk production. The current study can be adopted in selecting individuals with structures that are important in the characteristics of milk production and according to the genetic improvement plans to be applied.

Conclusion:

The study revealed a significant association between certain PITX2 gene mutations and milk production traits in goats. The GG genotype showed superior performance in milk season length and protein and fat content. Shami goats outperformed local ones in yield. These mutations are recommended for use in genetic improvement programs.

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