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Relationship Between Pitx2 Snps with Weights and Body Measurements in Goat

Shaimaa G. A. Al-Rubaye[1*], Zainab Hussein Kareem Al-Zubaidi[2], Huda Muhanna Redha[3], Ali Ahmed Kalaf[4]

[1,2,4]Department of Agricultural Biotechnology, Kut-Technical Institute, Middle Technical University, Iraq
[3]Al-Suwaira Technical Institute, Middle Technical University, Iraq

Email: shaimaa-ghawi@mtu.edu.iq

Abstract. This study was conducted at the Ruminant Research Station, affiliated with the Ministry of Agriculture, from December 1, 2021, to March 5, 2022. The objective was to investigate the relationship between single nucleotide polymorphisms (SNPs) in the Paired-like homeodomain transcription factor A (PITX1) gene and growth traits, including body measurements, in goats. A total of 52 female goats were used, including 16 native and 36 Shami goats. DNA sequencing of a 966 bp fragment from the promoter region of the PITX1 gene revealed four mutations. The SNPs G1198A and G1089A exhibited two genotypes: wild-type GG and heterozygous GA, with the mutant homozygous genotype AA not detected. For the C1194T SNP (referred to in the original text as ClYIT), three genotypes were identified: CC (wild), CT (heterozygous), and TT (mutant). Another SNP, G1003A, presented all three genotypes: GG, GA, and AA. The results showed that birth weight and body measurements, such as heart girth and body length, were significantly affected by the C1194T mutation and varied among genotypes. Most other growth traits, however, were not significantly influenced by breed differences. In detail, the highest dam birth weight was observed in the CC genotype (3.23 kg), while the CT genotype was associated with lower neonate birth weight (3.19 kg). The TT genotype (mutant) recorded the highest values for heart girth (83.4 cm) and body length (79.45 cm) in neonates. Furthermore, the G1003A mutation significantly increased dam birth weight, reaching 3.55 kg in individuals with the mutant AA genotype, although it had no significant effect on other growth traits or body dimensions.

Highlights:

1. PITX1 SNPs identified: Four mutations were found in the promoter region, notably C1194T and G1003A.
2. Growth traits affected: C1194T and G1003A SNPs significantly influenced birth weight and body measurements.
3. Genotype impact varies: TT and AA genotypes showed superior values in specific growth traits.

Keywords: Pitx2, Goat, Snps, Growth, Traits

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Introduction

The rapid increase in global population has led to a growing demand for essential resources such as food and medicine. To meet these needs, there has been an increasing reliance on modern agriculture and livestock farming, supported by advanced molecular biotechnology techniques. These technologies have significantly contributed to improvements in animal production, genome sequencing, and the development of genetic maps [1]

Production and reproductive traits in cattle are influenced by both genetic and environmental factors. Animals with superior production performance are prioritized in breeding programs aimed at genetic improvement. These programs depend on the genetic value passed from one generation to the next. Selection for genetic improvement is also guided by the heritability of multiple traits, where animals with higher genetic merit are expected to produce superior offspring [2]

Many economically important traits are controlled by genes that play critical roles in various biological functions. One such gene is the Paired-like homeodomain transcription factor 2 (PITX2), located on chromosome 6 in goats and cattle. As a transcription factor, PITX2 influences cellular characteristics and regulates the development of several organs, including the pituitary gland. Additionally, PITX2 is involved in the regulation of procollagen lysyl hydroxylase gene expression and contributes to the development of anatomical structures such as the eyes, teeth, and abdomen. It also plays a key role in establishing left-right body axis symmetry and the positioning of internal organs [3]

Moreover, PITX2 regulates the CVG gene, which is essential for embryonic development and twinning polarity [4]. It also activates the MYOD gene, which is responsible for muscle formation. The PITX2-MYOD pathway interacts with MYF5 and MYF6 genes to regulate muscle development [5]. The PITX1 gene has been strongly associated with growth and meat production traits [6]. It is located within a Quantitative Trait Loci (QTL) region related to meat production and is highly expressed in skeletal muscle.

Single nucleotide polymorphisms (SNPs) within this gene can affect meat quality attributes such as tenderness, juiciness, and fat deposition, and they also play a role in

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muscle mass development and body fat accumulation [7] [8]. Research by Zhao, X. et al, revealed that mutations in the first intron of the PITX gene are strongly associated with growth traits in cattle. Furthermore, mutations in the promoter region of the gene were found to correlate with growth performance in goats [9] [10]. The PITX gene genotype is significantly associated with growth and development via its influence on mRNA expression [11]

Goats are considered productive livestock animals due to their high reproductive efficiency, disease resistance, and adaptability. Therefore, this study aimed to investigate the effect of PITX gene polymorphisms on growth traits and body measurements in goats. In Iraq, several goat breeds are raised across different regions, each with varying production traits. Among these, the Shami goat is an introduced breed known for its high productivity under suitable environmental conditions [12]

Material and Methods

This study was conducted on 18 native and 36 Shami breed goats, aged between 1 to 6 years, at the Ruminant Research Station in Abu-Ghraib. The animals were housed in semi-open barns and managed under different feeding systems depending on the breeding plans. All animals were provided with some concentrated feed daily, while green forage was offered freely. Birth weights were recorded within two hours post-parturition using a specialized balance with a capacity of 20 kg. The kids were also weighed at weaning, around the age of 12 ± 15 days. Body weight gain was calculated as the difference between weaning weight and birth weight. Additionally, dams were weighed at parturition.

Body measurements were taken for each animal while standing on a flat surface. The heart girth was measured by wrapping a measuring tape around the chest just behind the front legs. The body length was measured from the point of the shoulder to the base of the tail. The wither height was defined as the vertical distance from the highest point of the scapula to the ground, while the rump height was the vertical distance from the highest point of the pelvic girdle to the ground [13]. Blood samples were collected from the jugular vein of 52 goats, comprising both native and Shami breeds. Laboratory work was carried out at the Molecular Genetics Laboratory in

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Baghdad, where DNA was isolated for the purpose of identifying mutations within the PITX1 gene.

1. DNA Extraction

A 5 mL blood sample was collected in tubes containing the anti-coagulant EDTA K2 and stored at 4°C. DNA extraction was performed as follows: 300 µL of blood was mixed with 900 µL of red blood cell (RBC) lysis buffer, followed by the addition of 20 µL of Proteinase K solution. The mixture was gently mixed for 10 minutes at room temperature to lyse the blood cells. The tubes were then centrifuged at 3000 rpm for 5 minutes, and the supernatant was carefully removed without disturbing the white blood cell layer. The mixture was vortexed, incubated at 60°C for 10 minutes, and mixed continuously every 3 minutes. Afterward, 200 µL of absolute ethanol was added to the tubes, and the mixture was vortexed gently.

The GD column was placed into 2 mL tubes containing the mixture, and the contents were centrifuged at 1400 rpm for 5 minutes. The supernatant was discarded, and 400 µL of Wash Buffer was added to the GD column. The tubes were centrifuged at 14,000 rpm for 30-60 seconds, and the supernatant was discarded. A second wash was done with 600 µL of Wash Buffer, centrifuged at 11,000 rpm for 30-60 seconds, and the supernatant was again discarded. Then, 100 µL of Elution Buffer was added to the GD column, which was placed in a water bath at 80°C for 3-5 minutes and centrifuged at 11,000 to 16,000 rpm for 30 seconds. The eluted DNA was stored at -20°C. The DNA concentration was measured using a Nanodrop spectrophotometer at a wavelength of 260 nm, with concentrations ranging from 29 to 49 ng/µL.

2. PCR Products Loading

5 mL of the sample was directly loaded into the wells. The electrical power was applied at 100 volts and 50 mA for 60 minutes. The DNA migrated from the cathode to the anode. Bromide-stained bands in the gel were visualized using a gel imaging system. PCR products were then sent for sequencing using the ABI 3730 XL automated DNA sequencer by Macrogen Corporation, Korea. The results were received via email and subsequently analyzed using Geneious software.

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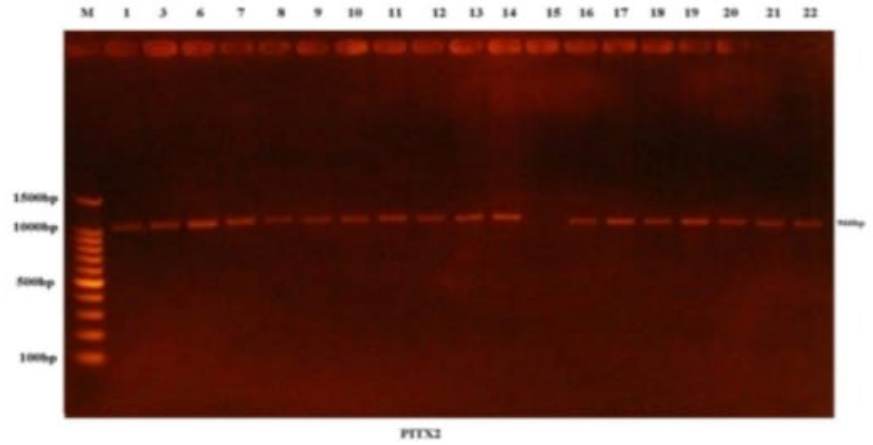
3. Primer Preparation

The primers were provided by Macrogen Company in a lyophilized form. The lyophilized primers were dissolved in nuclease-free water to achieve a final concentration of 10 µM/ml as a stock solution. A working primer solution was prepared by adding 10 µL of the primer stock solution (stored at -20°C) to 90 mL of nuclease-free water.

4. DNA Electrophoresis

Electrophoresis is used to verify the presence of DNA extracted from blood samples. To prepare the gel, dissolve 1 g of 10% agarose powder in 100 mL of TAE 1X buffer, heat in a microwave for 1.5 minutes until fully dissolved, and let the solution cool to 50-60°C. Add 1 µL of Ethidium Bromide stain (10 mg/mL) and mix. Prepare the casting mold by securing the plate edges and placing a comb to create wells, then pour the gel to avoid air bubbles and let it set for 10-30 minutes. Once the gel is hardened, remove the comb and place the gel in an electrophoresis tank filled with TAE 1X buffer, ensuring the gel is fully immersed. Mix 6 µL of DNA with 2 µL of Bromophenol Blue dye and load the mixture into the wells using a micropipette. Run the electrophoresis at 100 mA for 85 minutes, then transfer the gel to a UV light transilluminator to visualize the DNA bands, which will appear as bright orange spots due to the Ethidium Bromide dye. Capture the bands using a camera for further analysis.



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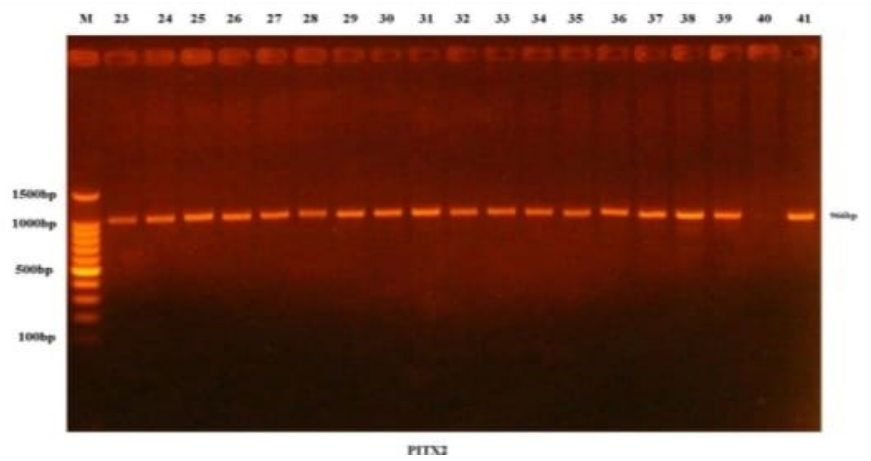

Figure 1. Results of the Amplification of PITX2 gene were Fractionated on 1.5% Agarose Gel Electrophoresis with Eth.Br.M:100bp Ladder Marker.

Statistical Analysis

Data was analysed by Statistical Analysis System for studying effect of genotypes of pitte gene within multi mutations on some of growth traits / Significant differences between means were compared through Duncan test and least square means was used according to next model [14]

Result and Discussion

A. Comparison between Native and Shami Goat in Growth Traits

There was no significant effect of breed on growth traits. However, Shami goats showed an increase in dam birth weight, weight at birth, and body length, which reached 2.20 kg, 2 kg, and 77.05 cm, respectively. On the other hand, native goats showed a higher weight gain in dams, reaching 18.65 kg, and had a rump height of 76.18 cm (Table 1). The results of this study contradict those of Jimmy, S. et al and Salim, A. H., who indicated that Shami goats exhibit significantly better growth traits compared to local goats across all dimensions. In contrast, the current research aligns with the findings of Salim, A. H., who reported that the dam birth weights for Shami and native goats were 3.15 kg and 2.86 kg, respectively, while their weaning weights were 15.22 kg and 15.89 kg. Moreover, body dimensions, such as rump height, were higher in this study, with Shami and native goats measuring 76.18 cm and 75.05 cm,

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respectively, compared to 52.61 cm and 53.15 cm recorded by Salim, A. H.. Variations in growth traits across studies may be attributed to the genetic potential for neonatal growth, as well as the relationship between increased body dimensions and body weight, which are linked to bone growth in neonates. Additionally, dams with higher weights tend to produce heavier neonates Jimmy, S. et al [15] [16]

Table 1. Comparison between Local and Shami Goats in the Characteristics Studied on Mothers and Newborns

Adjectives	**mean ± standard error**		**LSD**
	Domestic Breed Number =) (16	**Shami dynasty (Number = 36)**	
Mothers weight at birth (kg)	0.15± 2.75	0.11± 2.80	NS
Mothers weight at weaning (kg)	1.08± 21.31	0.78± 20.25	NS
Mothers weight gain between birth and weaning (kg)	0.97± 18.55	0.73± 17.45	NS
Mothers weight at birth(kg)	1.66± 39.06	1.17± 40.82	NS
Baby weight at birth (kg)	0.17± 2.85	0.12± 2.84	NS
The weight of the Calves at weaning (kg (kg)	1.41± 24.81	1.04± 24.05	NS
Bark weight gain between birth and weaning (kg)	1.42± 21.95	1.02± 21.21	NS
Chest Circumference (cm)	1.52± 81.75	1.46± 81.50	NS
Lbody length (cm)	1.43± 76.37	1.23± 77.05	NS
Height from front to front (cm)	1.21± 73.75	0.82± 73.50	NS
Height from back to front (cm)	1.14± 76.18	0.77± 75.05	NS
NS ، (P<0.05) *insignificant The averages with different letters within the same column differ significantly between each other			

1. Effect of Pitxr Genotypes within on Growth Traits in Goat G1148A Mutation

The G1148A mutation did not influence the growth traits of dams and their neonates. Higher growth values were observed in the wild AG genotype compared to the heterozygous GA genotype (Table 2). Dams with the AG genotype had a birth weight of 2.33 kg and produced neonates with a birth weight of 2.85 kg. The weaning weight for dams and neonates were 20.98 kg and 24.43 kg, respectively, coupled with higher weight gains of 18.12 kg for dams and 21.58 kg for neonates. In contrast, weight gains were reduced to 14.80 kg for dams and 20.16 kg for neonates in the GA genotype. Additionally, individuals

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with the GG genotype exhibited higher body dimensions compared to those with the GA genotype. This indicates that the G allele plays an important role in body growth, especially in the dominant genotype. The association between body weight and body dimensions, such as rump height, wither height, and heart girth, was significantly correlated at 0.590, 0.617, and 0.490, respectively [17] [18]

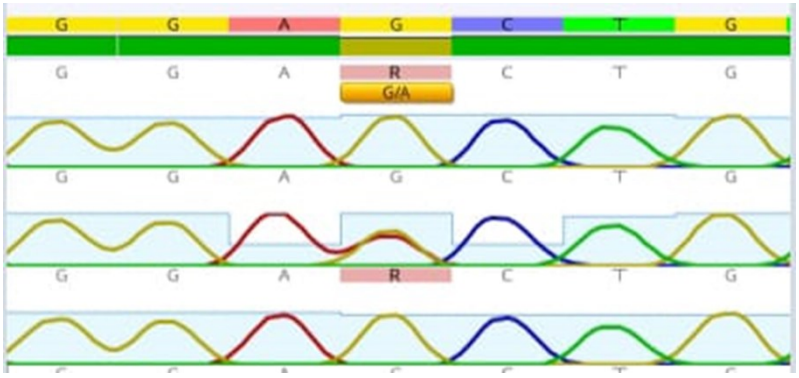

Figure 2. Analysis of G1148a Snp of Pitx2 Gene Using Sanger Sequencing. Single G Peak Indicative of A G Homozygous Allele. Presence of The G And A Peak Indicative of G / A Heterozygous Allele.

Table 2. Effect of PITX2 Genotypes Within The G1148A Mutation on The Studied Traits in Goats.

Adjectives	mean ± standard error		LSD
	GG	GA	
Mothers weight at birth (kg)	0.09± 2.83	0.17± 2.40	NS
Mothers weight at weaning (kg)	0.66± 20.95	1.46± 17.20	NS
Mothers weight gain between birth and weaning (kg)	0.61± 18.12	1.48± 14.80	NS
Mothers weight at birth(kg)	1.02± 40.63	2.56± 37.00	NS
Baby weight at birth (kg)	0.11± 2.85	0.28± 2.84	NS
The weight of the Calves at weaning (kg)	0.90± 24.43	2.16± 23.00	NS
Bark weight gain between birth and weaning (kg)	0.89± 21.58	2.05± 20.16	NS
Chest Circumference (cm)	1.18± 81.78	3.32± 79.60	NS
Lbody length (cm)	1.04± 77.06	1.24± 74.80	NS
Height from front to front (cm)	0.73± 73.65	0.96± 72.80	NS
Height from back to front (cm)	0.69± 75.48	1.60± 74.60	NS
NS ، (P <0.05) *insignificant The averages with different letters within the same column differ significantly between each other			

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2. Effect of Pitxa Genotypes within cll4IT Mutation on Growth Traits in Goat

The results of this study indicated that genotypes associated with the CLLALT mutation significantly impacted dam birth weight. The birth weight was 3.23 kg for the CC genotype, while it was 2.67 kg for CT and 2.75 kg for TT. Furthermore, neonate birth weights were influenced by different genotypes. The birth weight was 3.19 kg for the TT genotype, whereas it decreased to 2.57 kg for CC and 2.68 kg for CT. Additionally, the TT mutant genotype was predominant in terms of heart girth and body length for dams, which increased to 83.44 cm and 19.15 cm, respectively. In contrast, the measurements for CT were 79.83 cm for heart girth and 74.50 cm for body length, while for CC they were 78.85 cm and 74 cm, respectively. However, dam and neonate weights at birth, weaning, and weight gain were not significantly affected by the different genotypes (Table 3).

Regarding body dimensions, the study by [19] reported a minimum chest circumference of 54.25 cm and a body length of 52.86 cm. In comparison, the current study recorded higher body measurements. These findings align with those of [20] [21], who reported the lowest body length and body weight, which were 36.37 cm and 54.86 cm, respectively, whereas the current study recorded significantly higher measurements.

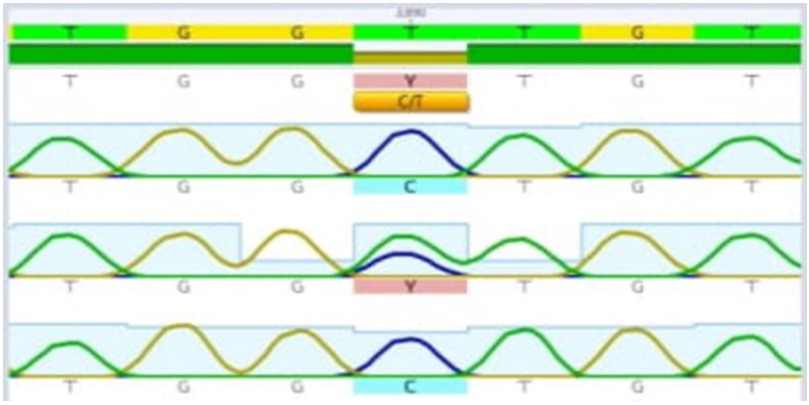

Figure 3. Analysis of C1141T SNP of PITX2 Gene using Sanger Sequencing. Single C Peak Indicative of A C Homozygous Allele. Presence of The C and T Peak Indicative of C / T Heterozygous Allele.

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Table 3. Effect of PITX2 Genotypes within the C1141T Mutation on the Studied Traits in Goats.

Adjectives	mean ± standard error			LSD
	CC	CT	TT	
Mothers weight at birth (kg)	3.23 a 0.21±	0.15± 2.67 b	2.75 b 0.11±	*
Mothers weight at weaning (kg)	21.00 1.41±	20.05 1.15±	20.84 0.89±	NS
Mothers weight gain between birth and weaning (kg)	17.77 1.24±	17.38 1.06±	18.09 0.84±	NS
Mothers weight at birth(kg)	40.28 0.99±	40.22 1.61±	40.31 1.52±	NS
Baby weight at birth (kg)	2.57 b 0.27±	0.16± 3.19 a	2.68 ab 0.12±	*
The weight of the Calves at weaning (kg)	23.42 1.13±	23.00 1.53±	25.42 1.20±	NS
Bark weight gain between birth and weaning (kg)	20.85 1.19±	19.81 1.51±	22.73 1.16±	NS
Chest Circumference (cm)	78.85 b 3.59±	79.83 ab 1.31±	83.44 a 1.68±	*
Lbody length (cm)	74.00 b 2.16±	74.50 b 0.92±	79.15 a 1.53±	*
Height from front to front (cm)	70.85 1.28±	73.55 0.95±	74.29 1.05±	NS
Height from back to front (cm)	73.28 1.51±	74.94 0.94±	76.25 0.97±	NS
NS ،(P <0.05) *insignificant The averages with different letters within the same column differ significantly between each other				

3. Effect of Pitxa Genotypes within G1089A Mutation on Growth Traits in Goat

Various genotypes of the Pitx2 gene did not have an effect on the weights and weight gain of dams and neonates, as well as body dimensions. Individuals with the GG genotype had a wither height of 74.20 cm and a rump height of 76 cm, while individuals with the GA genotype had a wither height of 76 cm and a rump height of 75.45 cm. Weight gain, heart girth, and body length reached 21.58 kg, 81.78 cm, and 77.06 cm, respectively, for the GG genotype. However, the GA genotype showed an increase in dam birth weight and weaning weight, which were 3.02 kg and 22.20 kg, respectively. Additionally, the weight gain for the dam was 19.18 kg, while the dam's weight at birth was 44 kg (Table 4).

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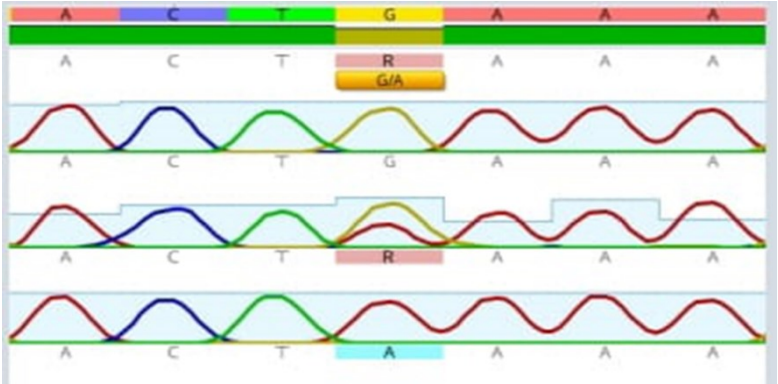

Figure 4. Analysis of G1089A SNP of PITX2 Gene Using Sanger Sequencing. Single G Peak Indicative of A G Homozygous Allele. Presence of The G and A Peak Indicative of G / A Heterozygous Allele.

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	
Mothers weight at birth (kg)	0.09± 2.75	0.29± 3.02	NS
Mothers weight at weaning (kg)	0.65± 20.44	2.78± 22.20	NS
Mothers weight gain between birth and weaning (kg)	0.60± 17.68	2.70± 19.18	NS
Mothers weight at birth(kg)	1.01± 39.78	3.49± 44.00	NS
Baby weight at birth (kg)	0.11± 2.84	0.29± 3.00	NS
The weight of the Calves at weaning (kg)	0.94± 24.33	1.46± 23.80	NS
Bark weight gain between birth and weaning (kg)	0.92± 21.49	1.47± 20.80	NS
Chest Circumference (cm)	1.22± 81.76	2.63± 80.80	NS
Lbody length (cm)	1.05± 77.06	1.52± 76.20	NS
Height from front to front (cm)	0.74± 73.63	1.11± 74.20	NS
Height from back to front (cm)	0.70± 75.45	1.14± 76.00	NS
NS ،(P <0.05) *insignificant The averages with different letters within the same column differ significantly between each other			

4. Effect of Pitxe Genotipes within G1003A Mutation on Growth Traits in Goat

The G1003A mutation affects dam birth weights ($p<0.05$), with mutant AA showing an increase to 3.55 kg, compared to 2.77 kg for genotype GG and 2.71 kg for GA. However, no significant differences were observed among the dams and their neonates in terms of other growth traits and body dimensions across the genotypes. Despite this, the wild GG genotype was predominant over

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GA in many of the growth traits. Animals with the AA genotype exhibited higher values for several traits, including weaning dam weight (22.50 kg), dam weight gain (18.95 kg), neonate weaning weight (25.50 kg), and weight gain (23.25 kg). The heart girth for these animals was 85 cm, and their body length was 75.50 cm (Table 5).

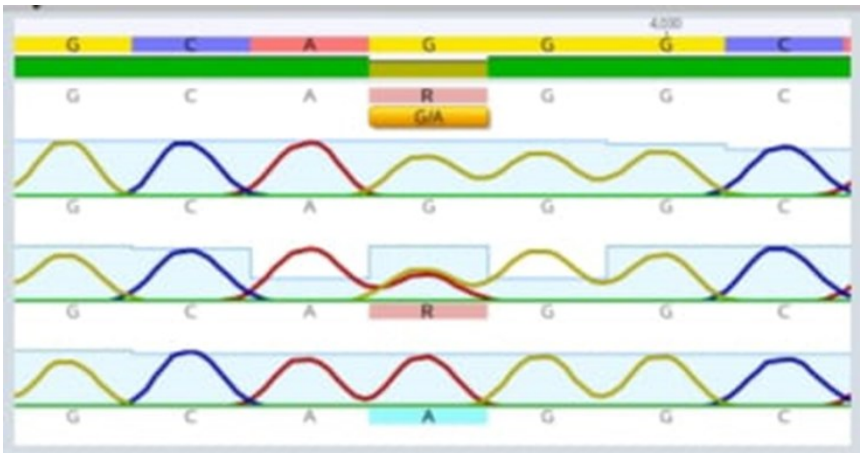

Figure 5. Analysis of G1003A SNP of PITX2 Gene Using Sanger Sequencing. Single G Peak Indicative of A G Homozygous Allele. Presence of The G and A Peak Indicative of G / A Heterozygous Allele.

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	
Mothers weight at birth (kg)	0.09± 2.77 b	b0.21± 2.71	0.45± 3.55 a	*
Mothers weight at weaning (kg)	0.69± 20.67	1.61± 20.00	3.50± 22.50	NS
Mothers weight gain between birth and weaning (kg)	0.64± 17.90	1.49± 17.29	3.05± 18.95	NS
Mothers weight at birth(kg)	1.23± 40.24	1.53± 39.83	0.50± 43.50	NS
Baby weight at birth (kg)	0.11± 2.87	0.23± 2.87	0.26± 2.25	NS
The weight of the Calves at weaning (kg)	0.99± 24.62	1.84± 23.08	0.50± 25.50	NS
Bark weight gain between birth and weaning (kg)	0.98± 21.75	1.77± 20.21	0.75± 23.25	NS
Chest Circumference (cm)	1.41± 82.29	1.18± 78.75	8.00± 85.00	NS
Lbody length (cm)	1.22± 77.73	0.95± 74.25	5.50± 75.50	NS
Height from front to front (cm)	0.83± 73.47	1.15± 74.33	3.00± 71.00	NS
Height from back to front (cm)	0.79± 75.42	1.04± 75.41	5.01± 75.00	NS
NS ، (P<0.05) *insignificant The averages with different letters within the same column differ significantly between each other				

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Conclusions

We concluded that individuals with the wild GG genotype in the G1148A mutation exhibit higher growth means compared to those with the GA genotype, although the genotype did not show a significant effect on growth traits. However, the Glosa A mutation, which includes the GA genotype, was predominant in dam birth weight, weaning weight, and weight gain, as well as the birth weight of their neonates. The wild GG genotype, on the other hand, showed higher weaning weight for neonates and greater weight gain for dams, along with larger heart girth and body length. Based on these findings, we recommend selecting animals with the GA genotype, as they demonstrate superiority in most growth traits.

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