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The Genetic Polymorphism Relationship of GDF9 Gene Mutations to Milk Production and its Components in Awassi Sheep

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Annotation: The study was conducted on 67 ewes and their offspring at the Khairat Al-Ittihad Sheep and Cattle Breeding Station in Babil Governorate, Iraq, from December 2023 to May 2024. Data were collected from the station's records, including ages, weights, and birth sequences, as well as laboratory analyses of blood and milk samples. This study aimed to investigate the genetic polymorphism of GDF9 gene mutations with milk production and milk components in Awassi sheep. DNA was extracted from blood samples using specialized techniques, and the target genes GDF9 and PRL were amplified using polymerase chain reaction (PCR). The results showed no nucleotide changes (mutations) in the GDF9 gene, while three mutations were identified in the PRL gene: SNP1: 277 C>T, SNP2: 684 G>T, and SNP3: 737 T>G. The allelic frequencies of the mutations were varied, with the CC genotype predominating in SNP1, the GT hybrid in SNP2, and TG in SNP3. Statistical results showed no significant differences in most of the studied traits between the different genotypes, with a statistically

significant superiority in total milk production observed for the GT genotype in SNP2.

Keywords: genetic polymorphism, GDF9 gene mutations, SNP1, SNP2, SNP3, milk production, components, Awassi sheep.

Introduction:

Sheep are an integral part of the agricultural economy in Iraq. Sheep farming is one of the oldest agricultural activities practiced by the Iraqi people, it has played a vital role in providing food and other by-products. Iraqi sheep are characterized by their genetic and ecological diversity, which reflects the country's great diversity of terrain and climate (Yousif *et al*., 2023). Sheep farming plays a vital role in the Iraqi economy, contributing significantly to the provision of meat, milk, wool, and leather. Many rural residents also rely on sheep farming as a primary source of income, which helps improve the standard of living in rural areas (Scoones and Webb, 2002).

Milk production in sheep is an important and vital aspect of agriculture and the global economy. It plays a major role in meeting human needs for a variety of food products. It is a rich source of protein, fat, vitamins, and minerals, making it highly nutritious. Milk yield varies depending on the type of sheep. Certain breeds, such as the Awassi sheep in the Middle East and the Lacon sheep in France, are known for their high milk yields. These breeds contain higher levels of fat and protein than cow's milk, with fat ranging from 6-8% and protein from 5-6% (Wang *et al*., 2022).

The basic components of milk include proteins, fats, carbohydrates, vitamins, and minerals. Proteins such as casein and lactoglobulin play a key role in the growth and health of muscle and skin tissue. Fat contributes to providing the energy needed for body activity. Carbohydrates, particularly lactose, are a primary source of energy and are easily digestible. In addition, sheep milk production has significant economic importance, as it directly contributes to supporting the local agricultural economy and providing job opportunities in related industries such as the dairy and cheese industry. Some countries are developing programs to improve local breeds and enhance their production capacities to improve the standard of living of farmers (Baltenweck *et al*., 2020; Mekuriaw *et al*., 2022). Various components of milk, such as calcium, phosphorus, and vitamins, contribute to promoting overall health and strengthening bones (Martin and Rosales, 2014).

Milk production in Iraqi sheep is affected by multiple factors, including nutrition, health care, and environmental conditions. Good, balanced nutrition containing protein- and fiber-rich feed contributes to increased milk production and quality. Proper health care and disease control are also essential factors for ensuring sustained productivity (Ahmed *et al*., 2019; Al-Hamdani *et al*., 2023). Environmental conditions such as high temperatures can negatively impact milk production, as heat stress can lead to decreased productivity, therefore, providing a suitable environment, adequate shade, and fresh water is essential for improving productivity (Al-Samarai and Al-Anbari, 2009).

The GDF-9 gene, which belongs to the TGF-β family, contains several exons and introns. The exons encode the GDF-9 protein, while the introns have a regulatory role. The gene includes protomer regions, which contribute to the regulation of gene expression. The gene typically consists of two to three exons. GDF-9 is an essential protein in the growth and development of

follicles in the ovaries of mammals. It contributes to the regulation of ovulation and increased fertility through interactions between ovarian cells. This protein plays a key role in communication between ovarian cells and promoting follicle growth, making it an essential component of reproductive functions (Lincoln, 2002; Souza *et al*., 2001).

The prolactin gene's genetic makeup consists of several exons and introns. Exons contain the sequences that encode the prolactin protein, while introns play a role in regulating gene expression, the gene also includes promotor regions that regulate when and where the gene is expressed. The prolactin gene typically consists of four to six exons connected by introns. Prolactin acts as a protein hormone secreted from the anterior lobe of the pituitary gland. It plays a key role in stimulating mammary cells to produce milk, in addition to regulating the immune system and promoting cellular growth and development (Souza *et al*., 2001; Spencer and Bazer, 2002).

Aims to demonstrate the relationship between the genetic polymorphism of GDF9 gene mutations and milk production and its components in Awassi sheep.

Materials and Methods:

This study was conducted at the Khairat Al-Ittihad Sheep and Cow Breeding Station, affiliated with the Al-Ittihad Company, in the Shomali District of Babil Governorate, from December 1, 2023 to May 31, 2024, it was conducted. Sixty-seven mother animals and 67 lambs were used for one production season. The parents ages ranged from 2 to 4 years. Data was obtained from the records maintained at the station, including their ages, birth numbers, birth weights, and current birth sequence. The laboratory aspect was also conducted in the station's laboratory.

Blood samples were drawn from the jugular vein in the neck using a 10 ml syringe. A total of 5 ml of blood was drawn for each sample after the blood collection area was cleaned and sterilized with ethyl alcohol. The samples were then emptied into a sterile test tube free of anticoagulants. They were stored at -4°C until laboratory use and DNA extraction.

DNA was extracted from sheep blood samples using a kit provided by the Korean company Geneaid.

Primers for the GDF9 gene were purchased by the Korean company Macrogen, in the form of a dried powder placed in a special tube labeled with the nitrogenous base sequence. The primers were prepared by adding 300 microliters of distilled water (dd water), to achieve a primer concentration of 100 picomoles. This is considered the stock solution, and 10 microliters were then taken from it. 90 microliters of distilled water (dd water) were added again. This resulted in a primer concentration of 10 picomoles, the concentration required for PCR. Table (1) shows the dilution of the primers and the quantities of distilled water (dd water) added.

Table (1) shows the dilution amounts for the primers.

Chemical Substance	Master Mix	DNA template	Primers		distilled water	Final volume
			Forward	Reverse		
Volume (microliter)	13	4	1	1	6	25

Table (2) Sequence of GDF9 gene primers used.

Gen	Primers	Volume/ nucleotide	GC%
GDF9-F1	5'- ACTGATGAATAAGGTTGTG - 3'	20	40
GDF9-R1	5'- ACTACTCATCATTAACTGCC - 3'	21	38
GDF9-F1	5'- GTTTTTGGTCAATTGAGGTTTAGAGC - 3'	24	42

GDF9-R2	5'- GGGCGGGGATTTCACACTGG - 3'	19	63

Table (3) Stages of PCR technique for GDF9 gene.

Gen	Stages	Temperatures	Time (min.)	Cycle No.
GDF9	First metamorphosis	95C°	5	1
	The Metamorphosis	95C°	0.30	35
	Adhesion	55 C°	0.30	
	Elongation	72C°	1.00	
	Final elongation	72C°	10	1

Results and Discussion:

The results showed that the first mutation, SNP1, for all genotypes (TT, CT, and CC), showed no significant differences in daily, weekly, or total milk production. However, there was a statistically significant superiority in total milk production for the CC genotype over the TT and CT genotypes. Total milk production was 56.81, compared to 51.62 and 56.41 for TT and CT. The second mutation, SNP2, showed no significant superiority in milk production for all genotypes. However, there was a statistically significant superiority in total milk production for the GT genotype over the GG and TT genotypes, with a value of 59.46, while GG and TT genotypes were 52.79 and 51.62, respectively. Regarding the third mutation, SNP3, no significant superiority in milk production was observed for all genotypes. However, the TT genotype was significantly superior in milk production over the GT and GG genotypes, with a value of 62.88, for GT and GG, the values were 51.92 and 51.76, respectively. In a study of 251 sheep, Abousoliman *et al*. (2020) indicated no significant difference between genotypes in total milk yield. However, AG (heterozygous) showed a significant superiority compared to GG and AA, similar to our results for SNP2 (Table 4).

Table (4) The effect of genotypes on daily, weekly and total milk production (mean + standard error).

Mutations	Genotypes	Daily milk production/liter	Weekly milk production/liter	Total milk production/liter
SNP1	**CC**	0.66 ± 0.04	4.69 ± 0.33	56.81 ± 4.07
	CT	0.64 ± 0.06	4.55 ± 0.43	56.41 ± 5.66
	TT	0.60 ± 0.07	4.22 ± 0.54	51.62 ± 5.95
SNP2	**GG**	0.60 ± 0.05	4.26 ± 0.36	52.79 ± 4.53
	GT	0.69 ± 0.05	4.91 ± 0.36	59.46 ± 4.59
	TT	0.60 ± 0.07	4.22 ± 0.54	51.62 ± 5.95
SNP3	**GG**	0.57 ± 0.07	4.02 ± 0.51	51.76 ± 7.11
	TG	0.60 ± 0.04	4.28 ± 0.30	51.92 ± 3.67
	TT	0.73 ± 0.06	5.19 ± 0.44	62.88 ± 5.66
Sig.		N.S	N.S	N.S

The results showed that the first mutation, SNP1, for all genotypes (TT, CT, and CC), showed no significant differences in milk components, including fat, solids, lactose, and protein. The second mutation, SNP2, also showed no significant superiority in milk components, including fat, solids, lactose, and protein, for all genotypes.

Regarding the third mutation, SNP3, no significant superiority in milk components, including fat, solids, lactose, and protein, for all genotypes. Ribeiro *et al.* (2019) found in a study conducted on

184 Assaf ewes that PRL mutations, did not show any significant effect on milk components, including fat, solids, lactose, and protein. They did not affect milk components. This is completely consistent with our results in this study for SNP1, SNP2, and SNP3 (Table 5).

Mutations	Genotypes	Fat %	Solids %	Lactose %	Protein %
SNP1	CC	5.17 ± 0.34	9.92 ± 0.28	4.44 ± 0.02	4.75 ± 0.22
	CT	5.03 ± 0.50	10.09 ± 0.40	4.45 ± 0.04	4.81 ± 0.34
	TT	3.51 ± 0.76	8.69 ± 0.82	4.36 ± 0.07	4.08 ± 0.52
SNP2	GG	5.14 ± 0.47	9.74 ± 0.39	4.40 ± 0.02	4.63 ± 0.30
	GT	5.11 ± 0.34	10.15 ± 0.27	4.47 ± 0.03	4.86 ± 0.23
	TT	3.51 ± 0.76	8.69 ± 0.82	4.36 ± 0.07	4.08 ± 0.52
SNP3	GG	5.99 ± 0.55	10.60 ± 0.41	4.46 ± 0.03	5.37 ± 0.36
	TG	4.95 ± 0.42	9.86 ± 0.35	4.41 ± 0.02	4.71 ± 0.27
	TT	4.59 ± 0.40	9.57 ± 0.36	4.45 ± 0.05	4.44 ± 0.27
Sig.		N.S	N.S	N.S	N.S

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