



Relationship Between Growth Hormone Gene Polymorphism with Milk Production and Its Components in Black Iraqi Goat

Muntaha Y. Yousief*, Jaafar M. Owaid & Raghdan H. Mohsin

Department of Animal Production, College of Agriculture, University of Basrah, Iraq

*Corresponding author email: M.Y.Y.: muntaha.yousief@uobasrah.edu.iq, J.M.O.:
jaffar.owaid@uobasrah.edu.iq, R.H.M.: raghdan.mohsin@uobasrah.edu.iq

Received 29th August 2023; Accepted 5th December 2023; Available online 31st December 2023

Abstract: Local black goat are raised for the purpose of milk production. The goal of the current study was to identify the growth hormone gene's single nucleotide polymorphism (SNPS). Additionally, the effect of the GH gene SNP on the milk qualities of Iraqi black goat was evaluated. DNA was extracted from blood samples of 28 goat. A segment with a length of 330 bp in the third intron and the fourth exon of the GH gene was analyzed. Bioinformatics software was used to analyze the results. The results showed the presence of three genotypes (TT, TC, CC) at position 263 of the studied plot with a frequency of 0.36, 0.21, and 0.43, respectively. The local breed was characterized by a decrease in observed heterozygosity (21.43%). However, the expected heterozygosity was 50.65%. Low heterozygosity may indicate the presence of inbreeding within the same herd. Haplotypes network revealed a genetic relationship between the local Iraqi goat and the Egyptian goat. As well as the existence of a genetic relationship at a lower level with the Indian goats. From Tajima's D test and Fu's Fs (1.547 and 1.428), the results led to a rise in inbreeding. In milk production, energy, and the amounts of protein. The analysis of the data using bioinformatics tools predicted the effects of identified the genotypes in which the amino acid was not altered, and this is where the mutation was found. There is a relationship between the genetic polymorphism of the growth hormone gene and the production of milk and its components. The CC genotype was superior in milk production, energy, and the amounts of protein and fat over the TC and CC combinations.

Keywords: Domestic goat, GH gene, Milk Yield, Single Nucleotide Polymorphism.

Introduction

Growth hormone (GH) gene is a functional and local candidate gene for milk production traits. It is a gene responsible for the expression of a mono-peptide consisting of 190 amino acids. GH secreted and synthesized in the anterior lobe of the pituitary gland. This hormone influence body composition, growth and development, mammary gland formation, and milk production and composition (Kojima *et al.*,

1999). Yardibi *et al.* (2009) stated that GH has a role in fat metabolism which contribute in body growth during suckling period. Growth hormone also affect feed circulation for milk yield. According the results obtained by Lukes *et al.* (1989) and Schlee *et al.* (1994); the increase in milk yield related with the advancement of growth hormone. In addition, it is evidence for the association between the genetic variation in the GH gene

and the concentration of growth hormone in the plasma. Given that the growth hormone is involved in the initiation and maintenance of lactation in mammals. GH gene can be used as a potential genetic marker that deserves study for its effect on milk production (Yardibi *et al.*, 2009).

Goat are used in many countries mainly for the production of meat. They are also used in the production of milk, especially in European countries. Most of the goat breeds (96%) worldwide are found in Asia and Africa and 4% in developed countries (Nguyen *et al.*, 2023).

There are two indigenous Iraqi goat breeds, the local black goat and Meriz (Yousif & Mohammed, 2022). The local black goat is a small black goat, originated in Iraq, is the most common breed. It spread in southern and central Iraq, the main goal of its breeding is milk production (Yousif *et al.*, 2011). Therefore, enhancing milk production of this breed is one of the most important purposes of genetic improvement.

On the other hand, identification of genetic polymorphisms is important in farm animal husbandry (Ruzina *et al.*, 2010; Al-Qasimi *et al.*, 2019; Jaffar *et al.*, 2019). Using RFLP, Valinsky *et al.* (1990) detected two alleles for the GH gene locus in goats and sheep (Al-Shuhaib *et al.* 2019). These alleles impact growth, metabolism, lactation, and reproduction (Mousavizadeh *et al.*, 2009). Yamano *et al.* (1988) described the sequence of a growth hormone gene in the goat pituitary gland that is 2544 bp long and consists of 4 introns and 5 exons (Lan *et al.*, 2007). GH polymorphism has been detected in goats by several investigators (Mousavizadeh *et al.*, 2009). Researchers have found an association between growth hormone genetic polymorphisms and milk

production (Kioka *et al.*, 1989; Falaki *et al.*, 1997; Moneva *et al.*, 2020). Some studies showed a highly significant correlation between the genotypes of this gene with milk yield and protein percentage in Portuguese Algarvia goats (Malveiro *et al.*, 2001). Pollott & Gootwine (2004) showed that GH gene adopted in selection to increase milk production and improve its components. They observed a significant correlation between GH genotypes with the daily and total milk production and some components of milk of Assaf sheep breed. Malveiro *et al.* (2001) analyzed exons 1–5 of the goat growth hormone (GH) gene by PCR-SSCP method in Algarvia goats. Their results showed that the F/F patterns of exon 4 and the A/A patterns of exon 5 correlated positively with milk production ($P < 0.05$). Marques *et al.* (2003) also studied exons 1–5 of the GGH gene and found an association of exons 2 and 4 and milk production in Serrana goats. It was found that exon 4 is more polymorphic than other exons in the GH gene.

The aim of this study was to describe the association between GH gene polymorphism with milk production and its components in Iraqi local black goat.

Materials & Methods

In this study, 28 black local goat from private herds in southern Iraq were used. Goat aged 3 years and at their second season of milk production. The biochemical part included measuring the chemical composition of the milk components. It was conducted in the laboratories of the College of Agriculture, University of Basrah.

Collect milk samples and measure its components

Milk samples were collected from 28 nannies at a rate of 60 ml for each goat. Milk components (fat, protein, lactose, solids not

fat (SNF) and total solids) were estimated once every two weeks throughout the six month of the study. The samples were collected from the morning milking. Samples were analyzed by EKOMIHK milk analyzer of Dutch origin. Energy in milk,

$$\begin{aligned} \text{Energy in milk } & \text{kcal.kg}^{-1} \\ & = 291.14 \text{ kcal.kg}^{-1} \times \text{kg milk} \\ & + 10,944 \text{ kcal.kg}^{-1} \times \text{kg milk} \\ & \times \text{fat\%} \end{aligned}$$

[Fat% is treated as its g.100^{-1} g value (Hall, 2023)].

Blood samples were collected from the jugular vein, at a rate of 2 ml for each animal. DNA extraction process was carried out using the DNA extraction kit (gSYNC™ DNA Extraction Kit) produced by the Korean company Geneaid. The primer, forward: 5' GGA AGG GAC CCA ACA ATG CCA '3 and reverse: 5' CTG CCA GCA GGA CTT GGA GC '3 primers were used (Malveiro *et al.*, 2001). The initial denaturation at 95°C for 5 min, 30 cycles of denaturation at 95°C for 30 s. The annealing at 60°C for 30 s and extension at 72°C for 30 sec. Followed by a final extension at 72°C for 5 min (Malveiro *et al.*, 2001). Samples of 20 microliters of DNA were sent to Yang Ling Tianrun aoka Biotechnology Company in China to obtain the true sequences. The sequencing process was performed for one strand of DNA, which is Forward, for the purpose of identifying genetic mutations. The sequence identity in the GenBank was examined after the findings were obtained by Blast search tool. It matched a variety of species, but in varying degrees. The sequences of each sample were manually aligned. Three-dimension structure of caprine GH was generated following the rules described by Al-Shuhaib (2019).

Statistical analysis

Using the statistical program SPSS (2019, Version 26). A complete randomized design (CRD) was utilized to analyze data. Means were compared using the Revised Least Significant Difference (RLSD) test within the program and according to the following mathematical model,

$$Y_{ij} = \mu + T_i + e_{ij}$$

where Y_{ij} represents the value of the observation for each trait, μ is common mean, T_i Effect of genotypes, e_{ij} represent the experimental error with mean=zero and a variance = $Q^2 e$

Results & Discussion

Polymorphism of GH gene

Utilizing nitrogen base sequence analysis technology and bioinformatics algorithms, the growth hormone gene sequences of local black goats were registered and accession numbers were obtained from the National Center for Biotechnology Information (NCBI).523895, 523896, 523897, 523898, 523899, LC523900. The results showed the presence of a genetic mutation in the studied region of the GH gene in Iraqi goat. T base was replaced by the C base at position 263. It represents position 627 of the fourth exon of the coding region of the GH gene. This mutation is considered one of the silent mutations, as it did not change any of the amino acids (phenylalanine) encoded by this gene. SNP at position 263 produced three genotypes (CC, CT and TT) as C mutated to T. These mutations are considered new and not registered in the GenBank, as shown in table (1) and figs. (1 and 2).

The change in the three-dimensional shape resulting from a change in an effective amino acid in turn changes the twist of the peptide chain, which potentially causes alter the

function of the protein. In the event of a missense mutation that changes the amino acid first, its polarity and twist differ from the

original (Figs. 1 and 2). As for other mutations, nothing happens.

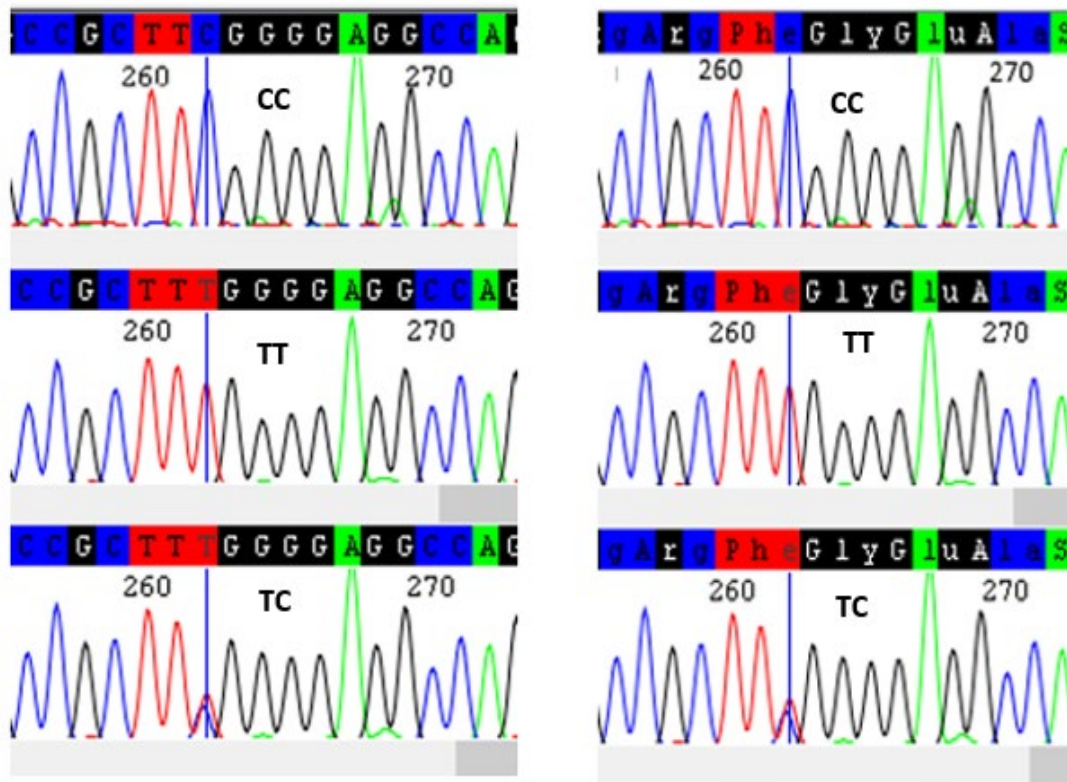


Fig. (1): Genotypes at the studied locus of the GH gene.

Table (1): Nucleotide changes and genetic codes for the growth hormone gene.

Location	Nucleotide	Code	Amino acid	Type of mutation
263	C>T	TTC>TTT	Phe>Phe	Synonymous

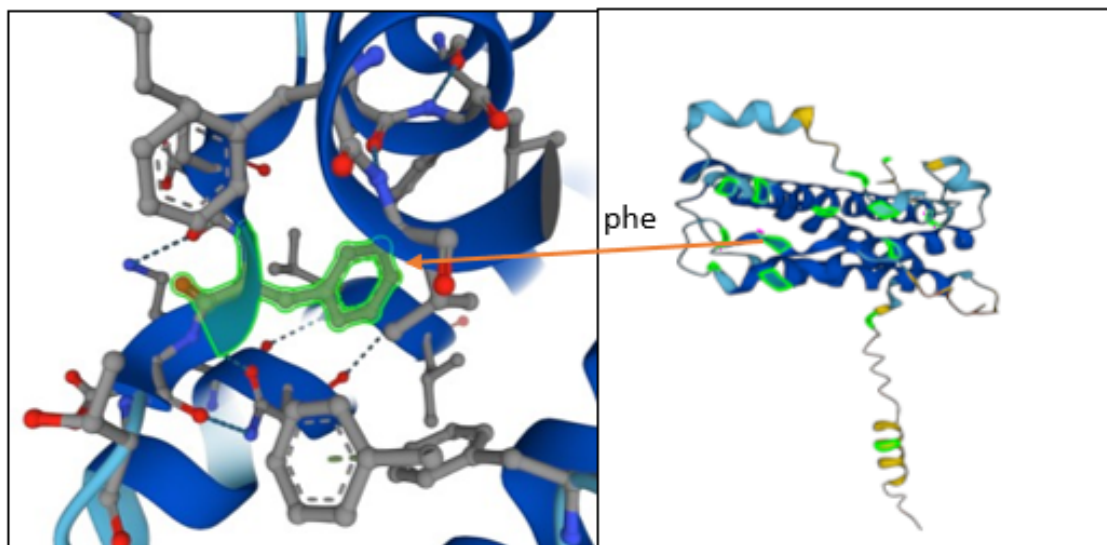


Fig. (2): Types of amino acid at the location of the mutations of GH gene.

CC genotype had the highest observed number (12) and the lowest (5.9091) expected number (Table 2). TC genotype had the lowest observed number (6) and the highest (14.1818) expected number. The number of animals with the TT genotype that were observed was 10, whereas the predicted number was 7.9091. Chi-square value was 9.668 ($P \leq 0.0018$), given that goats bred in very small herd sizes, this figure indicated random drift or inbreeding in this breed.

This value reflected inbreeding exists in this breed. It also revealed that Hardy equilibrium has not been reached. Gholamhoseinzadeh Gooki *et al.* (2018) discovered two genotypes in a sample of 300 Iranian goats, 255 and 45 animals were heterozygote and dominant genotype respectively. This result suggests outbreeding and their results disproved the current study's findings.

Table (2): The genotypes of the GH gene, observed and expected numbers, the χ^2 and p-value.

.Genotypes	Observed number	Expected number	χ^2	P value
TT	10	7.9091	9.668	0.001
TC	6	14.1818		
CC	12	5.9091		

Allele and Genotype Frequency and Fixation Index (Fis) for local black goats

There were three genotypes (CC, CT, TT) produced by two alleles, T and C, with a frequency of 0.46 and 0.54 respectively (Table 3). The frequency of the C allele is higher than the frequency of the T allele. The frequency of the TT, CT, and CC genotypes of the growth hormone gene were 0.36, 0.21, and 0.43, respectively. The highest frequency of genotypes is the CC genotype. The fixation index of the individual in the groups within the breed (Fis) for the two alleles was 0.5692.

This value indicates the presence of inbreeding, especially with the positive sign. Singh *et al.* (2015) found two genotypes in

the growth hormone gene in Indian Sirohi1 and Barbari2 breeds (AB and BB). The genotypic frequencies of AB and BB were found to be 0.82 and 0.18 in Sirohi and 0.90 and 0.10 in Barbari goats, respectively. Gholamhoseinzadeh Gooki *et al.* (2018) found two genotypes (AB and AA with a frequency of 0.15 and 0.85, respectively) for Iranian goats. Both studies indicated an outbreeding used in these areas, unlike the animals in this study.

Parameters of genetic variation of the growth hormone gene

The observed and expected heterozygosity (%) for the growth hormone gene in black local goats are shown in table (4). It shows n_a is two, n_e calculated by Kimura & Crow (1964) was 1.9898.

Table (3): Frequency of alleles and genotypes, and fixation index for black local goats.

Breed	NO	Allele Frequency	Genotype Frequency	Fis
-------	----	------------------	--------------------	-----

Local black goat	28	T	C	TT	TC	CC	0.5692
		0.46	0.45	0.36	0.21	0.43	

Shannon information index is one of the diversity indices used to measure frequency in the ordinal data (Lewontin, 1974). The fixation index appeared equal to 0.6906. Shannon's index (I) is also intended as the probability of symmetry to distinguish the difference of individuals (Paetkau *et al.*, 1995).

The heterozygote percentages observed and predicted were 21.43% and 50.65%, respectively. The presence of inbreeding within the same herd, which helps to raise the percentage of pure genotypes, may be represented by this low figure. Which is a reflection of the extent of gene flow (3.43%) that occurs when transmission of alleles occurs between the two groups. Consequently, there are few genotypes from outside the herd under study, and the inbreeding coefficient (0.6906) indicates a high level of inbreeding. There has been non-random mating, natural selection, mutations,

and genetic drift practice in this population. Non-random mating and natural selection disturb the population equilibrium through changes in gene frequency. Similarly, mutations interrupt allele frequencies by bringing new alleles into the population and genetic drift occurs in small populations. Moneva *et al.* (2020) who studied Anglo-Nubian dairy goats in the Philippines, the observed and expected heterozygosity of 0.47 and 0.76 indicates the presence of outbreeding and low inbreeding. Kunda *et al.* (2020) studied Lacor goats, they found that the observed heterozygosity was 50.2%. The frequency of the heterozygote genotype was 0.94 and the dominant was 0.06, as the Lacor goats in this population were in a state of genetic imbalance (HWE). The genetic imbalance in the population is influenced by several factors. Non-random mating associated with inbreeding, mutation, natural selection, genetic drift, migration, and gene flow (Hill & Mackay 2004).

Table (4): Parameters of genetic variation associated with the growth hormone gene in local black goats.

Breed	Number of alleles	na	ne	I	Observed heterozygosity	Expected heterozygosity
Local black goat	28	2.00	1.99	0.69	21.43%	50.65%

Haplotype’s network

The haplotypes network results show that there are six haplotypes (Fig. 3). The first haplotype included goats from Egypt, Iraq, and parts of the Indian goat breed. For the second haplotype, only goats from Egypt and Iraq were included. Only Egyptian, Indian, and Italian haplotypes 3, 4, 5, and 6 are found in these populations, respectively. There is a genetic connection between the indigenous

Iraqi goats and the Egyptian goats. However, a weaker connection between the

Iraqi goats and the Indian goats. This link arises from genuine migration or from the hybridization of an imported breed, whether Egyptian or Indian with an Iraqi breed. While the results did not show a relationship between the Iraqi strain and the Italian strains. Whereas, Al-Hassnawi & Ayied (2022a; 2022b) found that the haplotypes of local goats were shared with Bangladesh, China, India, Ireland, Italy, Russia, Turkey, and the United Kingdom. In contrary to what Owaid *et al.* (2023) found that the local black goat

differs from the global breeds, as it unique in its own genotypes.

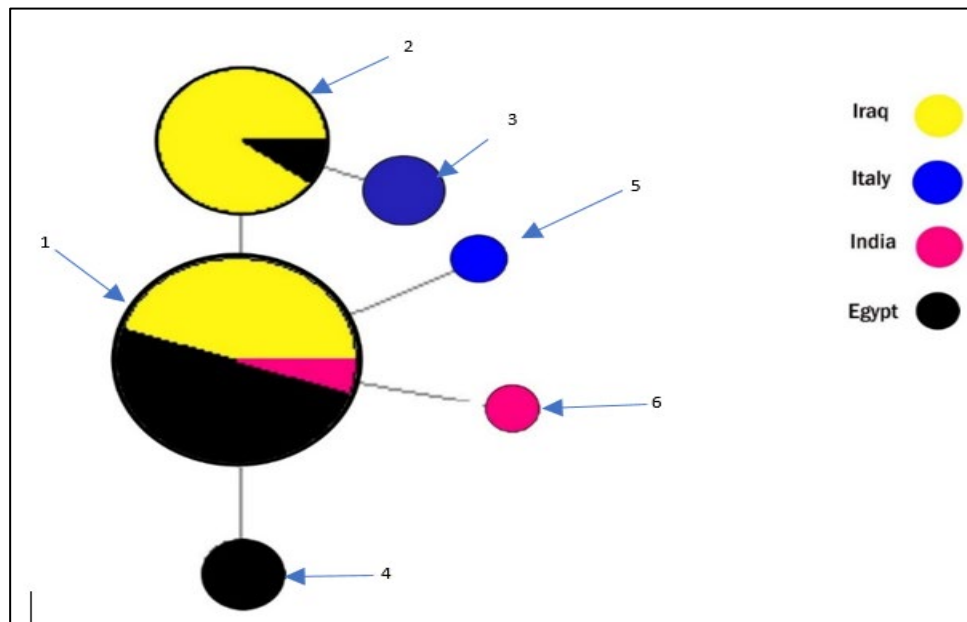


Fig. (3): The network of haplotypes of the Iraqi and some international breeds.

Neutrality Test

Fu's F_s and Tajima's neutrality test are used to calculate the likelihood that the population has experienced demographic events like genetic drift or population growth. The population is growing, as shown by the negative sign. The positive sign means that there are fewer people in the population. Genetic diversity declines as a result of individual loss, and structures and genetic variety also disappear. The results showed (Table 5) that there was no negative value, and the values were positive for each of Tajima's D test and Fu's F_s test (1.547 and 1.428). The growth hormone gene's results show that the breed is becoming more inbred and losing members. Small herd sizes, genetic drift, and the fixation of particular genotypes and alleles are the results. Coupled with the loss of many uncommon alleles. Along with causing the breed to approach a "bottleneck," these factors also significantly reduce the genetic variation in the breed. The reason for the positive values of the Tajima test for the studied gene may be due to the absence of organized scientific mating programs, or

selection, which may cause the loss of some genotypes (Tajima, 1989; Aris-Brosou & Excoffier, 1996; Fu, 1997). These findings corroborated those reported by Al-Hassnawi & Ayied (2022a; 2022b) in their investigation of two genes in Iraqi black goats.

Table (5) Neutrality test for the studied region of the growth hormone gene of black local goats

Breed	Tajima's D statistics	Fu's F_s statistics
Black local goat	1.547	1.428

Relationship between GH gene polymorphism with milk production and its components

The results of the current study (Table 6) showed that the variation in daily milk production according to the genotype of the GH gene was highly significant ($P \leq 0.01$). The highest daily, weekly, monthly, and total milk yields (1.093 and 7.65, 32.80 and 196.83 kg, respectively) were attained by the animals carrying the CC genotype. The TT genotype produced the least amount (0.716, 5.012, 21.48, and 128.88 kg, respectively). Between

the CC and TT combinations, females with the TC genotype produced 0.896, 6.276, 26.9, and 161.4 kg, respectively. High production is associated with the C allele. The behavior of the genotypes and alleles of the growth hormone gene in milk production can be inferred to be identical for all the variables examined. This outcome contrasts with that obtained by Malveiro *et al.* (2001) when they used the Portuguese Algarvia breed. This variation may result from genetic variations among breeds and genotypes, as well as from

variations in the study's methodology. According to Bekele *et al.* (2023), quantitative traits like milk production, composition, and growth are influenced by a large number of genes, each of which has a small effect, and each of which contributes a certain percentage to causing genetic variation. The difference in these results may be an indication of the weakness of the single effect of some genes on traits in isolation from other genes.

Table (6): Effect of genotypes of the GH gene on milk production ± SD.

Genotypes	No.	Milk Production (Kg)			Total
		Daily	Weekly	Monthly	
TT	10	0.72 ^c ± 0.05	5.01 ^c ± 0.32	21.48 ^c ± 1.37	128.88 ^c ± 8.23
TC	6	0.89 ^b ± 0.06	6.28 ^b ± 0.43	26.90 ^b ± 1.86	161.40 ^b ± 11.13
CC	12	1.09 ^a ± 0.04	7.65 ^a ± 0.287	32.81 ^a ± 1.23	196.83 ^a ± 7.37

*Means with different superscript in each column differ significantly at P≤ 0.05

Additionally, there was a linear and significant relationship (P< 0.05) between the yield of milk and the genotype of a growth hormone gene when the relationship between

each genotype and daily milk production was plotted (Fig. 4). The contribution of genotypes to the variation in daily milk production was 99.01%.

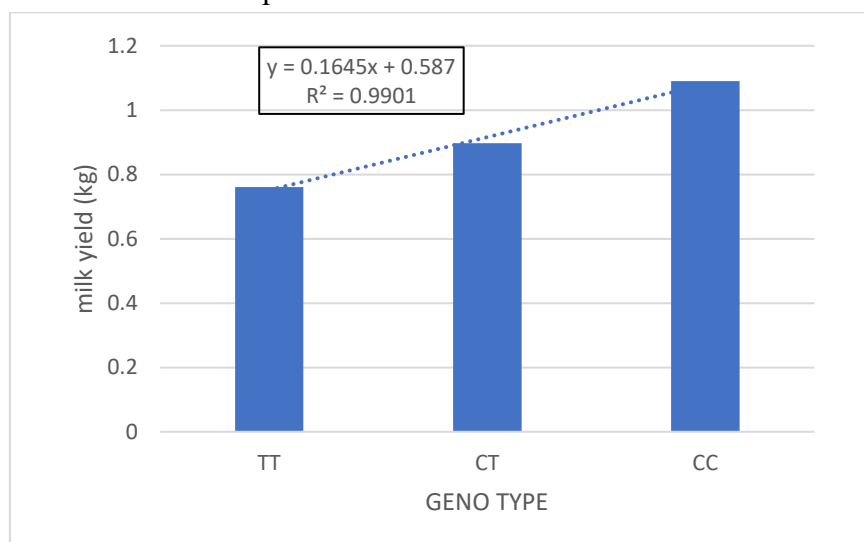


Fig. (4): Linear relationship between daily milk production and genotypes of the growth hormone gene.

It reflects the accuracy of prediction of total production from knowing the genotypes, as there was an increase in the amount of milk production with the genotype, the highest production was for the CC genotype and the lowest for the TT genotype. The average increase in the amount of milk production with genotype was about 0.1645 kg.

The results shown in table (7) show that there is a significant difference ($P \leq 0.01$) between the genotypes of the GH gene for milk energy, percentage of fat, amount of fat and protein, and percentage of SNF and total solids. The CC genotype was superior to the two genotypes TC and TT for milk energy (1.40, 43.55 and 47.38, respectively). Whereas, animals carrying the TT genotype outperformed the TC and CC genotypes in fat, SNF and total solids%. The TT genotype recorded 4.99, 14.35, and 10.19, respectively.

This result is similar to what Al-Salihi *et al.* (2017) found in terms of the absence of a significant variation in the percentage of protein according to the genetic structures of

the growth hormone gene in sheep, as there was a slight decrease in the percentage of protein with the change of genetic structures and then a slight increase, as well as a small decrease in the percentage of fat with the genetic structure and then a significant ($P \leq 0.05$) rising.

The percentage of fat is one of the most important structural characteristics of milk that determines the quality of milk, its price and the type of product from which it is made. Thus, the adoption of gene expression in improving this trait seems useful through the results of this study.

As there is a direct relationship between the percentage of fat and each of the percentage of SNF and total solids. The behavior of the genotypes and alleles of the studied gene is different, as the genotype CC showed different behavior than the genotypes TT and TC in the studied traits. The genotype of the growth hormone gene did not show any significant difference for both protein and lactose.

Table (7): Effect of GH gene polymorphism on milk components \pm SD.

Genotypes	No.	Milk composition							
		Milk energy $\text{kg}^{-1} \cdot \text{kcal}$	Fat%	fat (gm)	Protein %	protein (gm)	Lactose %	Total solid %	SNF %
TT	10	0.92 ^c	4.99 ^a	35.70 ^c	4.25 ^a	30.60 ^c	4.35 ^a	14.35 ^a	10.19 ^a
		\pm 0.06	\pm 0.09	\pm 1.73	\pm 0.51	\pm 5.30	\pm 0.26	\pm 0.626	\pm 0.855
TC	6	1.15 ^b	4.49 ^b	40.21 ^b	4.14 ^a	37.05 ^b	3.96 ^a	13.88 ^{ab}	9.41 ^b
		\pm 0.08	\pm 0.08	\pm 2.23	\pm 0.37	\pm 3.66	\pm 0.29	\pm 0.004	\pm 0.063
CC	12	1.40 ^a	3.98 ^c	43.55 ^a	4.38 ^a	47.83 ^a	4.35 ^a	13.33 ^b	8.69 ^c
		\pm 0.05	\pm 0.13	\pm 2.34	\pm 0.45	\pm 4.00	\pm 0.21	\pm 0.451	\pm 0.644

• Means with different superscript in each column differ significantly at $P=0.05$

Fig. (5) shows the relationship between milk energy, fat and protein percentage, and the genotypes of the growth hormone gene.

The milk energy measured in $\text{Kcal} \cdot \text{kg}^{-1}$ showed a linear relationship with the genotype with a high accuracy of 99.94%. There was a rise in the amount of milk energy

with the genotype, and the highest energy was for the CC genotype and the lowest for the TT genotype. The mean increase in the amount of energy in milk with the genotype was about 0.24 Kcal.kg⁻¹. This rise is usually associated with milk production.

Additionally, fig. (5) illustrates the linear relationship between the percentage of fat and genotype. This figure presents a slight decline in the percentage of fat with genotype, with an average decline in the percentage of fat of 0.505%, and the accuracy of estimating the percentage of fat based on the genotype was extremely high, confirming the type of linear relationship between the percentage of fat and genotype. This aids in highly accurate fat percentage change prediction for various genotypes.

The nature of the association between protein and GH genotypes is depicted in fig. (5). The graph demonstrates the existence of a quadratic non-linear relationship between them. Although the protein percentage somewhat decreased after the genotype alteration and then slightly increased, the difference was not statistically significant.

With a 100% contribution rate, the genotype change resulted in an average increase in the protein percentage of 0.105. Additionally, despite the fact that there is an inverse correlation between the volume of milk produced and the quantity of fat and protein in it, the rate of decrease in the percentage of fat and protein remained consistent. This demonstrates the potential for forecasting a decline in the proportion of fat and protein in the milk.

The relationship between the genetic conformation of the growth gene and the percentage of protein, fat, and energy in milk confirms the results of the analysis of variance and the significant effect of the genetic conformation of the growth hormone gene on milk production and its components (Moneva *et al.*, 2020). These results are consistent with Bekele *et al.* (2023). Which confirms that this gene has a positive relationship with increasing and improving fat and protein in cow's milk. The positive correlation of GH gene and either fat or protein% in milk was also determined in other species such as goats (Marques *et al.*, 2003) and sheep (Pollott & Gootwine, 2004).

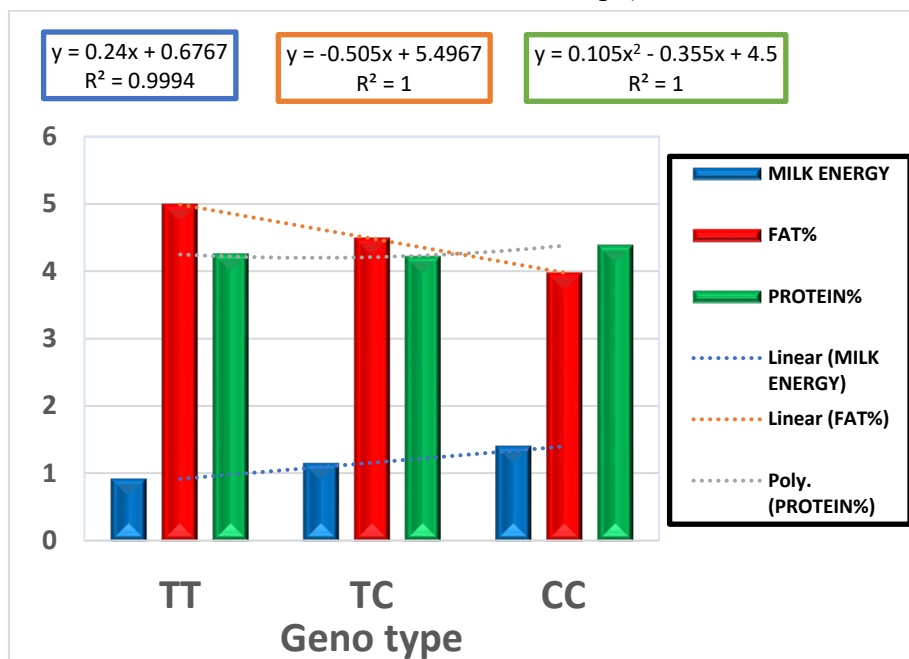


Fig. (5): The relationship between milk energy, fat%, protein%, and the genotypes of the GH gene.

The relationship between milk production and its components

Through correlation coefficients, the relationship between milk production and its components can be clarified. Fig. (6) shows that there is a positive and highly significant correlation ($P \leq 0.001$), as it ranged from 0.81 to 0.98 between each of milk production and each of the amount of fat, protein, and milk energy. By increasing the production, the amount of fat and protein increases, and by increasing them, the energy in the milk increases, because the energy depends on both the fat and the protein present in the milk. On the other hand, the correlation was negative and significant ($P \leq 0.001$) between each of the percentage of fat and the percentage of SNF and total solids with each of the milk production and the amount of fat, protein and energy, whose value ranged between -0.48 to -0.85. That is, the higher the milk production,

the lower the percentage of fat, because the relationship between them is inverse. This result agreed with what was found by Todaro *et al.* (2005), where he found a negative correlation between the percentage of fat and protein and milk production.

The result also agreed with what Al-Hubaety & Al-Juwari (2021) found that the correlation coefficient between milk production and the ratio of fat, protein, lactose and solids was negative and significant ($P \leq 0.01$), while the percentage of fat was positively and significantly associated with protein, lactose and solids not-fat, and they confirmed that high milk production leads to a reduction in the percentage of fat. As the correlation coefficient between two characteristics reflects the strength and direction of the relationship between them, which has a benefit in indirect selection processes to improve some qualities that are delayed in their appearance on the animal.

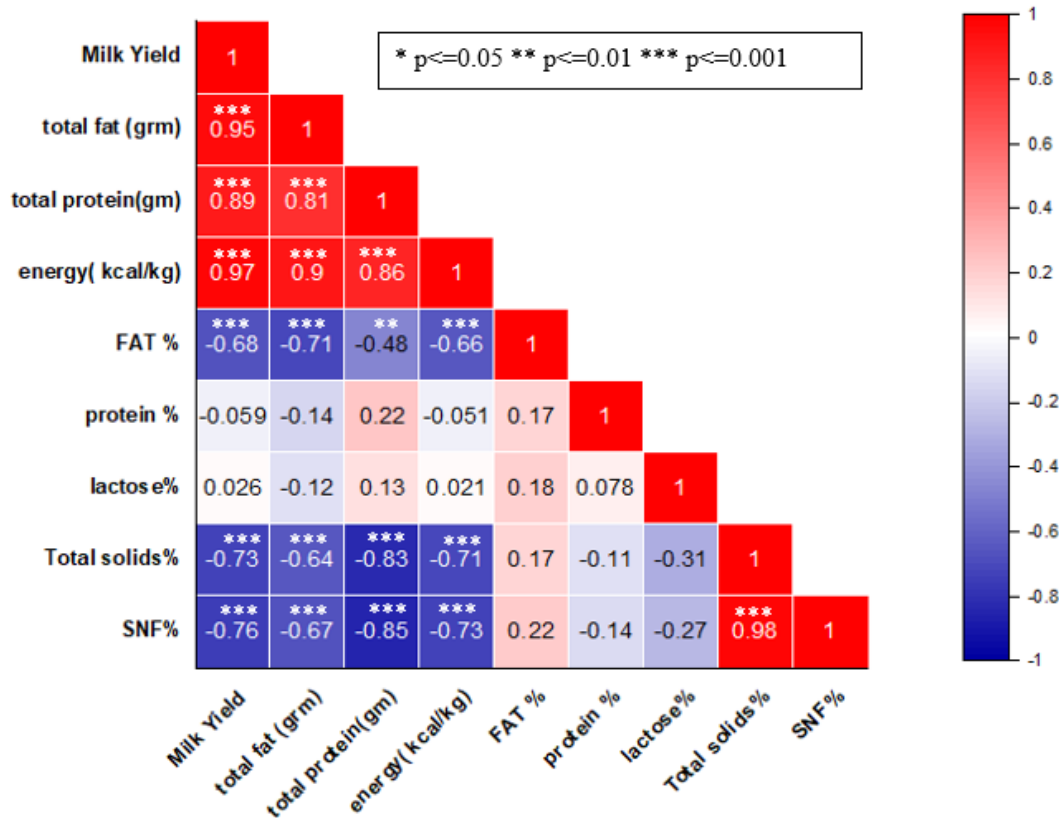


Fig. (6): Correlation coefficients between milk production and its components.

Conclusion

The results showed the presence of three genetic polymorphisms (TT, TC, CC) at position 263 of GH gene. Low levels of heterozygosity (21.43%) were observed, which suggests inbreeding. The haplotype network data showed a genetic link of a lower degree with the Indian goats and between the native Iraqi goats and the Egyptian goats. The amino acid's polarity and twist remained the same after the mutation; it did not alter initially. The CC genotype had higher amounts of protein and fat, produced more milk, and had more calories than the TC and CC genotypes. The GH gene is a useful marker that can be applied in a selection program to increase goat milk yield and its constituent.

Acknowledgements

The authors acknowledge the support of staff of Department of Animal Production, College of Agriculture, University of Basrah for conducting the research work.

Contributions of authors

M.Y.Y.: Milk samples collection, laboratory methodology, statistical analysis and writing part of the manuscript.

J.M.O., Milk samples collection, suggest a title of the manuscript and writing part of the manuscript.

R.H.M., Evaluation and writing part of the manuscript.

ORCID

M.Y.Y.: <https://orcid.org/0000-0002-1951-7371>

J.M.O.: <https://orcid.org/0000-0001-5893-7953>

R.H.M.: <https://orcid.org/0000-0002-4007-5774>

Conflicts of interest

The authors declare that they have no conflict of interests.

Ethical approval

All applicable national and international guidelines for the care and use of animals were followed.

References

- Al-Hassnawi, I. A. S., & Ayied, A. Y. (2022a). Detection of genetic diversity of local Iraqi black goats by using Cytochrome b gene. *NeuroQuantology*, 20(8), 7626-7634. https://www.neuroquantology.com/open-access/Detection+of+genetic+diversity+of+local+Iraqi++black+goats+by+using+Cytochrome_4019/
- Al-Hassnawi, I. A. S., & Ayied, A. Y. (2022b). Detection of genetic diversity of local Iraqi black goats by using D-loop Region. *NeuroQuantology*, 20(8), 7642-7651. https://www.neuroquantology.com/open-access/Detection+of+genetic+diversity+of+local+Iraqi++black+goats+by+using+D-loop+Region_4022/
- Al-Hubaety, A. K., & Al-Juwari, M. F. (2021). The effect of milking methods on milk production and its components and the relationship between milk components and biochemical characteristics of blood in Awassi sheep. *Journal of Agricultural, Environmental and Veterinary Sciences*, 5(2), 15-27. <https://journals.ajsrp.com/index.php/jaevs/article/view/3809>
- Al-Qasimi, R. H., Hassan, A. F., & Khudair, B. Y. (2019). Effect of IGF-1 and GH genes polymorphism on weights and body measurements of Awassi lambs in different ages. *Basrah Journal of Agricultural Sciences*, 32(1), 39-46. <https://doi.org/10.37077/25200860.2019.125>
- Al-Salihi, A., Al-Saadi, B., & Al-Anbari, N. (2017). Genotypes relationship of Growth hormone gene polymorphism with some productive and reproductive trait in Awassi sheep. *Journal of Biotechnology Research Center*, 11(2). (In Arabic) <https://doi.org/10.24126/jobrc.2017.11.2.516>
- Al-Shuhaib, M. B. S. (2019). A comprehensive in silico prediction of the most deleterious missense variants in the bovine LEP gene. *Journal of*

Biotechnology, Computational Biology and Bionanotechnology. 100(4C), 429-439.
<https://doi.org/10.5114/bta.2019.90244>

- Al-Shuhaib, M. B. S., Al-Thuwaini, T. M., Fadhil, I. A., & Aljubouri, T. R. S. (2019). GHRL gene-based genotyping of ovine and caprine breeds reveals highly polymorphic intronic sequences in Awassi sheep with several RNA motifs. *Journal of Genetic Engineering and Biotechnology*, 17(3), 1-8.
<https://doi.org/10.1186/s43141-019-0004-5>
- Aris-Brosou, S., & Excoffier, L. (1996). The impact of population expansion and mutation rate heterogeneity on DNA sequence polymorphism. *Molecular biology and evolution*, 13(3), 494-504.
<https://academic.oup.com/mbe/article/13/3/494/1057318>
- Bekele, R., Taye, M., Abebe, G., & Meseret, S. (2023). Genomic regions and candidate genes associated with milk production traits in Holstein and its crossbred cattle: A review. *International Journal of Genomics*, 2023, 1-18. <https://doi.org/10.1155/2023/8497453>
- Falaki, M., Prandi, A., Corradini, C., Sneyers, M., Gengler, N., Massart, S., Fazzini, U., Burny, A., Portetelle, D., & Renaville, R. (1997). Relationships of growth hormone gene and milk protein polymorphisms to milk production traits in Simmental cattle. *Journal of Dairy Research*, 64(1), 47-56. <https://doi.org/10.1017/s0022029996001872>
- Fu, Y.-X. (1997). Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics*, 147(2), 915-925. <https://doi.org/10.1093/genetics/147.2.915>
- Gholamhoseinzadeh Gooki, F., Mohammadabadi, M., & Asadi Fozi, M. (2018). Polymorphism of the growth hormone gene and its effect on production and reproduction traits in goat. *Iranian Journal of Applied Animal Science*, 8(4), 653-659.
https://ijas.rasht.iau.ir/article_544783.html
- Hall, M. B. (2023). Invited review: Corrected milk: Reconsideration of common equations and milk energy estimates. *Journal of dairy science*.
<https://doi.org/10.3168/jds.2022-22219>
- Hill, W. G. & Mackay, T. F. C (2004). D. S. Falconer and Introduction to Quantitative Genetics, *Genetics*, 167(4), 1529-1536. <http://doi.org/10.1093/genetics/167.4.1529>
- Jaffar, A. A., Hassan, A. F., & Kassim, W. Y. (2019). Effect of pou1fl gene haplotypes on eights and milk production of awassi sheep. *Basrah Journal of Agricultural Sciences*, 32(2), 85-94.
<https://doi.org/10.37077/25200860.2019.199>
- Kimura, M., & Crow, J. F. (1964). The number of alleles that can be maintained in a finite population. *Genetics*, 49(4), 725. <https://doi.org/10.1093/genetics/49.4.725>
- Kioka, N., Manabe, E., Abe, M., Hashi, H., Yato, M., Okuno, M., Yamano, Y., Sakai, H., Komano, T., & Utsumi, K. (1989). Cloning and sequencing of goat growth hormone gene. *Agricultural and Biological Chemistry*, 53(6), 1583-1587. <https://doi.org/10.1080/00021369.1989.10869514>
- Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Matsuo, H., & Kangawa, K. (1999). Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature*, 402, 6762, 656-660. <https://doi.org/10.1038/45230>
- Kunda, R. M., Volkandari, S. D., Rumanta, M., & Kakisina, P. (2020). Polymorphism of growth hormone (GH) gene in lakor goat from Lakor Island of Southwest Maluku regency. *Buletin Peternakan*, 44(4), 233-238. <https://doi.org/10.21059/buletinpeternak.v44i4.58934>
- Lewontin, R. C. (1974). *The genetic basis of evolutionary change*. Columbia University Press, New York, 346pp. <https://doi.org/10.1086/288688>
- Lukes, A., Barnes, M., & Pearson, R. (1989). Response to selection for milk yield and metabolic challenges in primiparous dairy cows. *Domestic Animal Endocrinology*, 6(4), 287-298.
[https://doi.org/10.1016/0739-7240\(89\)90023-4](https://doi.org/10.1016/0739-7240(89)90023-4)
- Malveiro, E., Pereira, M., Marques, P., Santos, I., Belo, C., Renaville, R., & Cravador, A. (2001). Polymorphisms at the five exons of the growth hormone gene in the algarvia goat: possible association with milk traits. *Small Ruminant Research*, 41(2), 163-170.
[https://doi.org/10.1016/S0921-4488\(01\)00198-5](https://doi.org/10.1016/S0921-4488(01)00198-5)
- Marques, P., Pereira, M., Marques, M., Santos, I., Belo, C., Renaville, R., & Cravador, A. (2003). Association of milk traits with SSCP polymorphisms at the growth hormone gene in the Serrana goat. *Small Ruminant Research*, 50(1-2), 177-185. [https://doi.org/10.1016/S0921-4488\(03\)00104-4](https://doi.org/10.1016/S0921-4488(03)00104-4)
- Moneva, C. S. O., Vega, R. S., Sangel, P. P., Angeles, A. A., & Mendiolo, M. S. (2020). Genetic variability in the growth hormone gene (A781G)

- and its association with milk yield performance in crossbred anglo-nubian dairy goats. *Philippine Journal of Science*, 149(3).
<https://www.ukdr.uplb.edu.ph/journal-articles/508/>
- Mousavizadeh, A., Mohammad Abadi, M., Torabi, A., Nassiry, M. R., Ghiasi, H., & AliEsmailizadeh Koshkoieh, A. (2009). Genetic polymorphism at the growth hormone locus in Iranian Talli goats by polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP). *Iranian Journal of Biotechnology*, 7(1), 51-53.
https://www.ijbiotech.com/article_7064.html
- Nguyen, V. D., Nguyen, C. O., Chau, T. M. L., Nguyen, D. Q. D., Han, A. T., & Le, T. T. H. (2023). Goat production, supply chains, challenges, and opportunities for development in Vietnam: A Review. *Animals*, 13(15), 2546.
<https://doi.org/10.3390/ani13152546>
- Owaid, J. M., Yousief, M., Abdulrda, A., & Ayied, A. (2023). Study of local black Iraqi goats genotypes for the cytb gene. *Archives of Razi Institut*, 78(3), 915-921. <https://doi.org/10.22092/ari.2022.359888.2499>
- Paetkau, D., Calvert, W., Stirling, I., & Strobeck, C. (1995). Microsatellite analysis of population structure in Canadian polar bears. *Molecular Ecology*, 4(3), 347-354. <https://doi.org/10.1111/j.1365-294X.1995.tb00227.x>
- Pollott, G., & Gootwine, E. (2004). Reproductive performance and milk production of Assaf sheep in an intensive management system. *Journal of dairy science*, 87(11), 3690-3703. [https://doi.org/10.3168/jds.S0022-0302\(04\)73508-0](https://doi.org/10.3168/jds.S0022-0302(04)73508-0)
- Ruzina, M., Shtyfurko, T., Mohammadabadi, M., Gendzhieva, O., Tsedev, T., & Sulimova, G. (2010). Polymorphism of the BoLA-DRB3 gene in the Mongolian, Kalmyk, and Yakut cattle breeds. *Russian Journal of Genetics*, 46, 456-463.
<https://doi.org/10.1134/S1022795410040113>
- Schlee, P., Graml, R., Schallenberger, E., Schams, D., Rottmann, O., Olbrich-Bludau, A., & Pirchner, F. (1994). Growth hormone and insulin-like growth factor I concentrations in bulls of various growth hormone genotypes. *Theoretical and Applied Genetics*, 88, 497-500. <https://doi.org/10.1007/BF00223667>
- Singh, P., Tomar, S., Thakur, M., & Kumar, A. (2015). Polymorphism and association of growth hormone gene with growth traits in Sirohi and Barbari breeds of goat. *Veterinary World*, 8(3), 382-387. *Abstract Aim: The aim was to study the polymorphism of exon, 2.* <https://doi.org/10.14202%2Fvetworld.2015.382-387>
- SPSS (2019) IBM Statistics 26 step by step. 16th Edition. <https://doi.org/10.4324/9780429056765>
- Tajima, F. (1989). Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics*, 123(3), 585-595.
<https://doi.org/10.1093/genetics/123.3.585>
- Todaro, M., Scatassa, M. L. & Giaccone, P. (2005). Multivariate factor analysis of Girgentana goat milk composition. *Italian Journal of Animal Science*, 4(4), 403-410.
<https://doi.org/10.4081/ijas.2005.403>
- Valinsky, A., Shani, M., & Gootwine, E. (1990). Restriction fragment length polymorphism in sheep at the growth hormone locus is the result of variation in gene number. *Animal Biotechnology*, 1(2), 135-144.
<https://doi.org/10.1080/10495399009525736>
- Yamano, Y., Oyabayashi, K., Okuno, M., Yato, M., Kioka, N., Manabe, E., Hashi, H., Sakai, H., Komano, T., & Utsumi, K. (1988). Cloning and sequencing of cDNA that encodes goat growth hormone. *FEBS letters*, 228(2), 301-304.
[https://doi.org/10.1016/0014-5793\(88\)80020-6](https://doi.org/10.1016/0014-5793(88)80020-6)
- Yardibi, H., Hosturk, G. T., Paya, I., Kaygisiz, F., Ciftioglu, G., Mengi, A., & Oztabak, K. (2009). Associations of growth hormone gene polymorphisms with milk production traits in South Anatolian and East Anatolian Red cattle. *Journal of Animal and Veterinary Advances*, 8(5), 1040-1044.
<https://medwelljournals.com/abstract/?doi=javaa.2009.1040.1044>
- Yousif, A. N., & Mohammed, A. (2022). Genetic diversity of Iraqi local goat breeds by RAPD DNA markers. *Jurnal Kedokteran Hewan*, 16(4), 127-131. <https://doi.org/10.21157/j.ked.hewan.v16i4.28579>
- Yousif, A. N., Mohammed A. K., Mahmmud B. M., & Juma F. T. (2011). Effect of parturition and lactation on some haematological and biochemical characteristics in Mountain Black goat. *Journal of Kirkuk University for Agricultural Sciences*, 2(1):57-65.
https://kujas.uokirkuk.edu.iq/article_33395.html

العلاقة بين تعدد أشكال جين هرمون النمو وإنتاج الحليب ومكوناته في الماعز العراقي الأسود

منتهى يعقوب يوسف، جعفر محمد عويد ورغدان هاشم محسن

قسم الإنتاج الحيواني، كلية الزراعة، جامعة البصرة، العراق

المستخلص: أجريت الدراسة الحالية لمعرفة تأثير تعدد أشكال النيوكليوتيدات المنفردة (SNPS) في جين هرمون النمو على صفات حليب الماعز المحلي الأسود إذ تم استخلاص الحامض النووي منقوص الأوكسجين (DNA) من عينات الدم لـ 28 معزة. ضخمت قطعة بطول 330 زوج قاعدي في الانترون الثالث والاكسون الرابع لجين GH باستخدام تقانة تتابع القواعد النيروجينية للكشف عن التغيرات الوراثية وحللت النتائج باستخدام برامج المعلوماتية الحيوية أظهرت نتائج الكشف عن وجود ثلاثة أشكال وراثية (TT,TC,CC) في الموقع 263 من القطعة المدروسة إذ بلغ تكرار التراكيب لجين هرمون النمو 0.36 و0.21 و0.43 على التوالي. أما نسبة الخلط الأليلي المشاهد فقد امتازت السلالة المحلية بانخفاض هذه النسبة وكانت قيمتها %21.43 ونسبة الخلط الأليلي المتوقع % 50.65 مما يعني انخفاض التراكيب الوراثية الخليطة أي وجود تزاوج داخلي ضمن القطيع الواحد الذي يعمل على زيادة نسبة التراكيب الوراثية النقية. ومن ناحية أخرى ومن النتائج المتحصل عليها من شبكة الأنماط الفردية يظهر ان هناك علاقة وراثية بين الماعز العراقي المحلي والماعز المصري كذلك وجود علاقة وراثية بمستوى اقل بين الماعز العراقي والماعز الهندي ويمكن القول ان هذه العلاقة هي نتيجة وجود الهجرة او الانتقال او ناتجة من الاختلاط بين السلالات العراقية وبعض السلالات المستوردة. ومن اختبار Tajima's D و Fu's Fs (1.547 و 1.428) تشير النتائج الى ان العشائر تخسر عدد من افرادها مما قد تؤدي الى حصول ارتفاع في الترتيب الداخلية، كما تبين النتائج ان هناك علاقة بين الاشكال الوراثية لجين هرمون النمو وإنتاج الحليب ومكوناته. إذ نفوق التركيب الوراثي CC في إنتاج الحليب والطاقة وكميتي البروتين والدهن على التركيبين TC وCC.

الكلمات المفتاحية: الماعز المحلي، جين GH، محصول الحليب، تعدد أشكال النيوكليوتيدات المنفردة.