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MOLECULAR DETECTED OF GHRELIN RECEPTOR GENE IN IRAQI MALE GOATS

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Abstract: Goats are vital livestock for milk, meat, and hair production across Asia and Africa, yet their genetic improvement has often been neglected. The growth hormone secretagogue receptor (GHSR) gene, critical in regulating metabolism and productivity through ghrelin signaling, is of particular interest for enhancing livestock performance. This study aimed to investigate the polymorphisms of the GHSR gene in Iraqi local goats. Eighteen male goats were sampled at the College of Agriculture, University of Basrah. DNA was extracted, amplified via PCR, and sequenced for analysis. The results revealed two novel genetic variants of the GHSR gene not previously recorded in global databases, showing 99.45% similarity to the San Clemente goat sequence (USA). Multiple sequence alignment demonstrated distinct mutations, including both silent and missense mutations, suggesting potential functional impacts on gene expression and protein function. These findings highlight the genetic diversity of Iraqi goats and suggest that GHSR polymorphisms may serve as valuable molecular markers for future breeding and productivity enhancement programs. Further research is needed to associate these genetic variations with physiological and productive traits.

Keywords: Iraqi goats, gene polymorphism, molecular detected, ghrelin receptor gene, molecular markers.

INTRODUCTION

Goats are considered one of the most important sources of milk, meat, and hair production in several regions of Asia and Africa. Despite this importance, goats have not received adequate attention for their development and improvement. They are often blamed as one of the major causes of forest and shrubland degradation and as contributors to desertification. The number of goats in the Arab world is approximately 85.718 million heads, distributed variably among Arab countries. In Iraq, around 300,000 heads are raised by breeders across different regions (Al-Dabbagh et al., 2012).

Ghrelin is one of the key hormones that plays a major role in regulating appetite and energy metabolism in living organisms. It was first discovered in 1999 and is known for its stimulatory effects on the secretion of growth hormone (GH) from the pituitary gland. Ghrelin is primarily secreted from the stomach but is also found in other tissues such as the intestines, pancreas, and brain. In ruminants such as cattle, sheep, and goats, ghrelin and the regulation of its levels play a critical role in physiological processes like digestion, metabolism, and milk production, the physiological effects of ghrelin are mediated through its binding to its specific receptor, known as the Growth Hormone Secretagogue Receptor (GHSR), which belongs to the G protein-coupled receptor (GPCR) family (Smith et al., 2005).

The ghrelin receptor plays a pivotal role in mediating ghrelin signaling within the body, and the gene encoding this receptor has garnered significant attention in genetic and physiological research due to its critical influence on animal performance and productivity. Current studies aim to elucidate the genetic and adaptive mechanisms that enable ruminants to regulate metabolic processes, particularly under conditions of nutritional or environmental stress (Van den Top et al., 2005; Zebeli & Metzler-Zebeli, 2012).

The polymorphisms represent one of the key phenomena contributing to biological diversity among individuals within different species. In the case of the growth hormone secretagogue receptor (GHSR) gene, polymorphisms can influence its function and expression levels, thereby impacting the physiological and productive traits of ruminants by affecting metabolism and energy balance. Genetic variations within the GHSR gene can lead to differences in the body's response to ghrelin signaling (Groeneveld et al., 2010).

These genetic variations may influence appetite regulation, energy utilization, and digestive efficiency, which subsequently affects growth and the production of milk or meat in ruminants. Additionally, there is a relationship with nutritional stress, as some studies suggest that polymorphisms in the GHSR gene are associated with the ability of ruminants to adapt to nutritional stress. For instance, certain mutations may enhance nutrient utilization efficiency during periods of food scarcity. Furthermore, these polymorphisms can impact productivity traits, as studies have shown that GHSR gene polymorphisms can affect productive traits such as milk quantity and quality in cows, or growth rate and weight gain in sheep and goats (Sirini et al., 2018; Habib et al., 2022).

Some genetic variants may be associated with an enhanced body response to ghrelin, leading to improved productive performance. Additionally, polymorphisms in the GHSR gene can have an impact on health and immunity. It is believed that certain mutations may influence the ability of the immune system to respond to environmental stress or diseases. Thus, GHSR gene polymorphisms could potentially affect the overall

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health status of ruminants, as some mutations may alter immune response mechanisms to stressors or infections (Galochkin et al., 2018; Habib et al., 2021).

In applied genetic studies, the analysis of GHSR gene polymorphisms has become an important tool in breeding programs. By identifying mutations associated with desirable traits, genetic techniques can be used to enhance the productive performance and adaptive capacity of ruminants (Li & Gao, 2017; Fathala & El-Magd, 2020). In the field of animal breeding, understanding the genetic factors influencing growth and productivity is crucial. Therefore, the study of the GHSR gene in Iraqi local goats is of significant importance, as genetic variation in this gene could contribute to the improvement of productive traits such as growth rates, feed efficiency, and disease resistance (Zhou et al., 2011). Given the limited studies on this gene, the aim of this study was to investigate the ghrelin receptor gene in Iraqi local goats.

METHODS AND MATERIALS

In the Department of Animal Production, College of Agriculture, University of Basrah, Basrah, Iraq, this study has been conducted (longitude 47.7433690, latitude 30.5627250 north of Basrah). 18 male goats between the ages of 5-6 months were used, for the period from 29/10/2023 to 2/2/2024. All animals were in good health, free of disease, and under constant veterinary supervision.

Samples collection and DNA extraction

Blood samples were collected from male Iraqi goats using EDTA tubes via the jugular vein at a rate of 10 mL. The samples were then transported to the laboratory and stored at −20°C until DNA extraction could be performed. DNA was isolated using the technique described by Högberg et al., (2022), using the (QIGEN) kit and according to the procedure the manufacturer advises. Using a Nanodrop, the concentration and purity of DNA were calculated.

The amplification of PCR

The amplification reaction was adopted with a volume of 25 µl for goat male as it consisted of 9.5µl water nuclease-free, 12.5 µl of 2X PCR master mixes, 1.0 µl (10 µM) of each primer (Forward and reverse),1.0 µl (75 ng) DNA template. Primers for the ghrelin receptor gene (GHSR) were designed according to the published sequence to amplify a region within the gene's coding DNA (CDS). This region was chosen because it corresponds to a portion of the open reading frame (ORF) responsible for encoding the ghrelin receptor protein. Mutations in this region can lead to amino acid substitutions, potentially affecting the protein's structure and function, and thus allowing for the identification of possible functional variants. The primer in goats was F-CAGGCGGATTTCAACTCCCT, R-CCTCTCACCGAGCCACTTTT (Kõressaar et al., 2018). The PCR conditions were 98 °C (initial denaturation) for 5 min pursued by 1 cycle, after that, 35 cycles of denaturation for 15 sec at 98º C, then the annealing was done for 30 sec at 55° C, the extension was 30 sec at 72 °C, the final extension was carried out for 5 min at ambient 72°C then pursued by 1 cycle. A 15% ethidium bromide stain was utilized (0.5 µg/ml agarose gel) to detect the PCR product, 3 Kb DNA ladder was used, the purification was done by following the manufacturer's instructions when using a Qiagen kit (Germany).

The analysis of Sequences

Sequence analysis has been completed in Malaysia's first BASE Laboratory, and the BLAST analysis and the Multiple Sequence Alignment have been done (Boratyn et al., 2019). The results of the Sequence were compared with the highest match rate in the NCBI to detect the possible molecular change (The comparison was in goatswith accession number XM_005675309, which represents goats san clemente in USA). To uncover the 3D structure of protein the Swiss model (Waterhouse et al., 2018) has been used, Mega software was used to analyse the phylogenetic tree (Tamura et al., 2021).

RESULT

Revealed that the GHSR gene in Iraqi local goats does not match any other record in the gene bank by 100%, due to several different mutations. The highest sequence similarity was with the San Clemente goat (USA) (accession number XM_005675309), at 99.45%. This indicates that the sequences of this gene identified in the current study represent entirely new sequences, never previously recorded globally (noting that this gene has not been directly registered in the gene bank, and all previous entries were based on predicted sequences).

Upon conducting multiple sequence alignment (MSA) analysis, two distinct genetic variants of the GHSR gene were obtained due to the presence of different mutations (Table 1). It is worth noting that these two genetic variants did not completely match (100%) due to different mutations, although some common mutations were observed (Fig. 1).

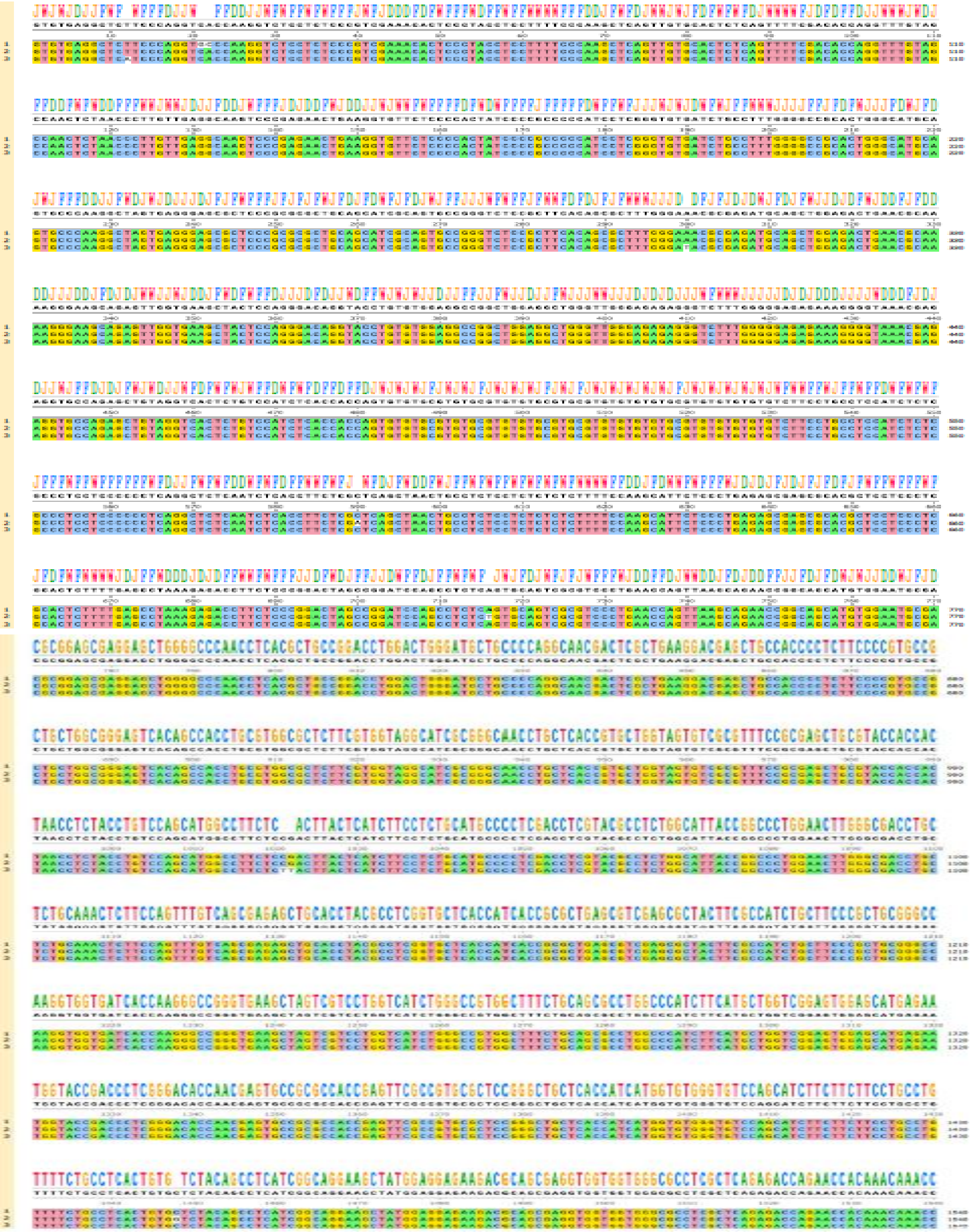
These polymorphisms were recorded in GenBank under accession numbers LC810420 and LC810421 (Fig. 2 A and B) .

It is important to note that the mutations that occurred in both polymorphisms obtained in the current study were silent, and others were missense mutations (Fig. 3).

On the other hand, the results of the study indicate the presence of a number of mutations (whether silent or notice missense) in different locations that were repeated in both genetic formations (21, 22, 1546, 1547, 1548, and 2127), which indicates that these animals are likely of common origin (Hoelzel and Lynch 2023).

Silent and missense mutations can also affect the expected three-dimensional shape of the resulting protein as well as the folding process (Fig. 4).

Protein function is directly related to its shape (Zhang et al., 2022). The folding process is also very important as it also affects protein function and resistance to various stress conditions (Durosaro et al., 2023).



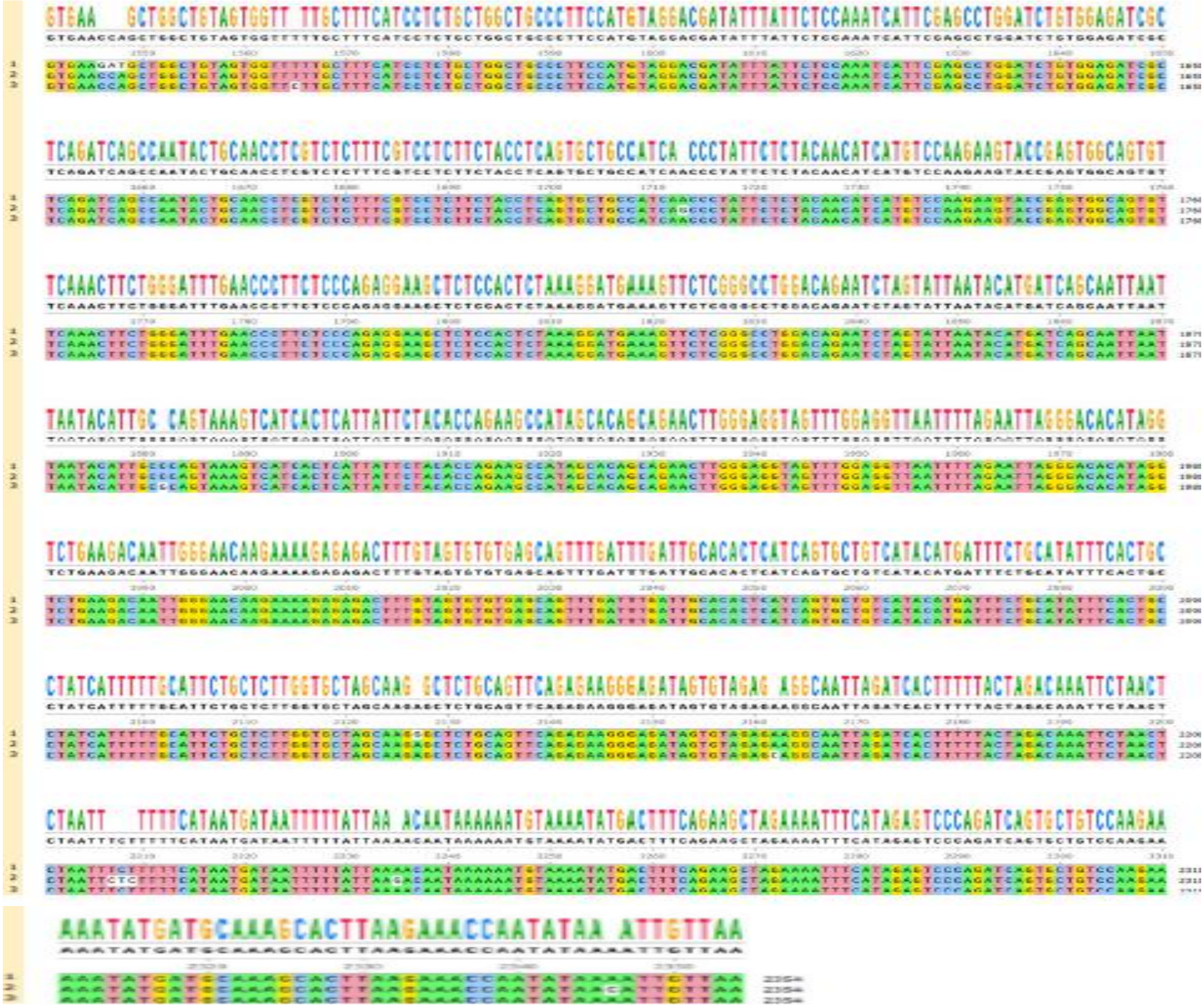
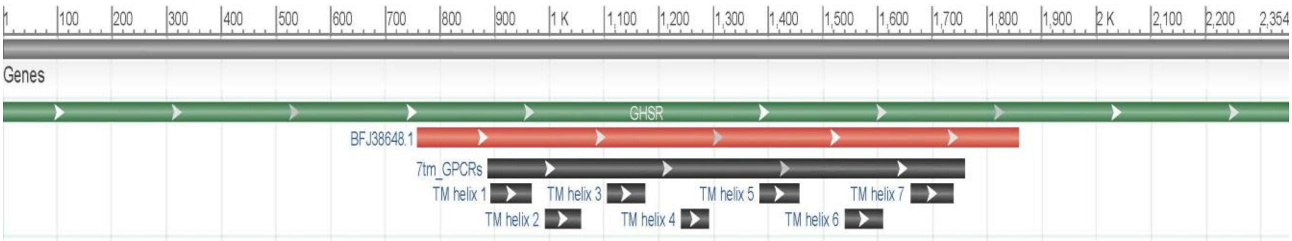


Fig. 1. Multiple Sequence Alignment for GHSR gene nucleotides in Iraqi Goat with reference gene.



A LC810420

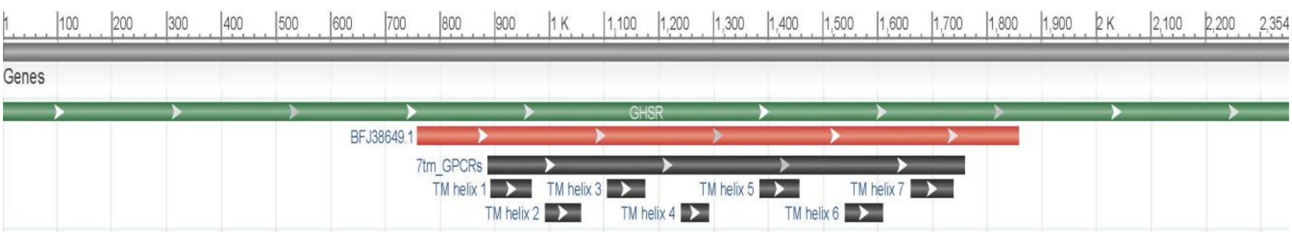


Fig. 2. Gene of the ghrelin receptor in local Iraqi goats.

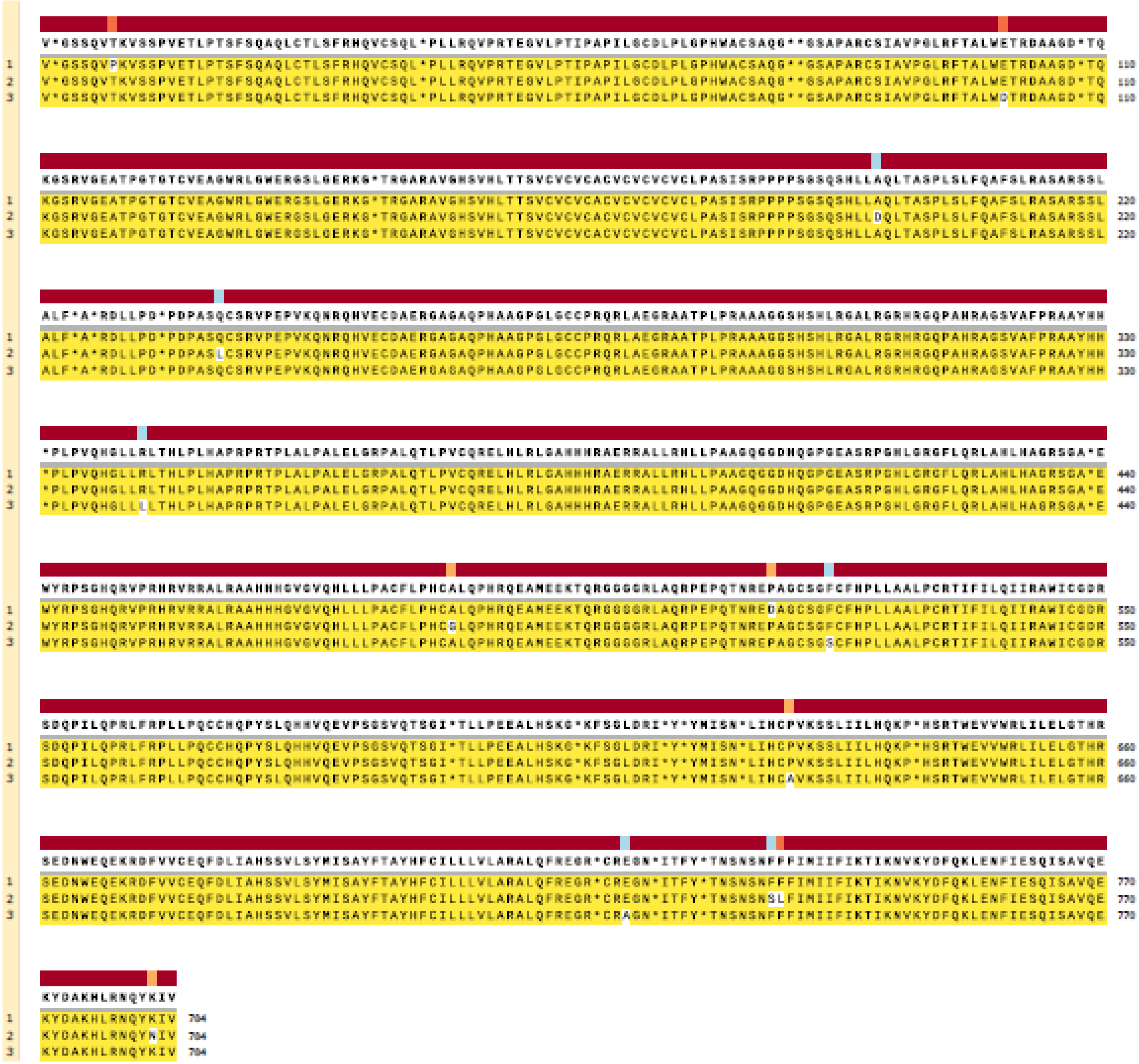


Fig. 3. Multiple Sequence Alignment for GHSR gene amino acids in Iraqi Goat with reference gene.

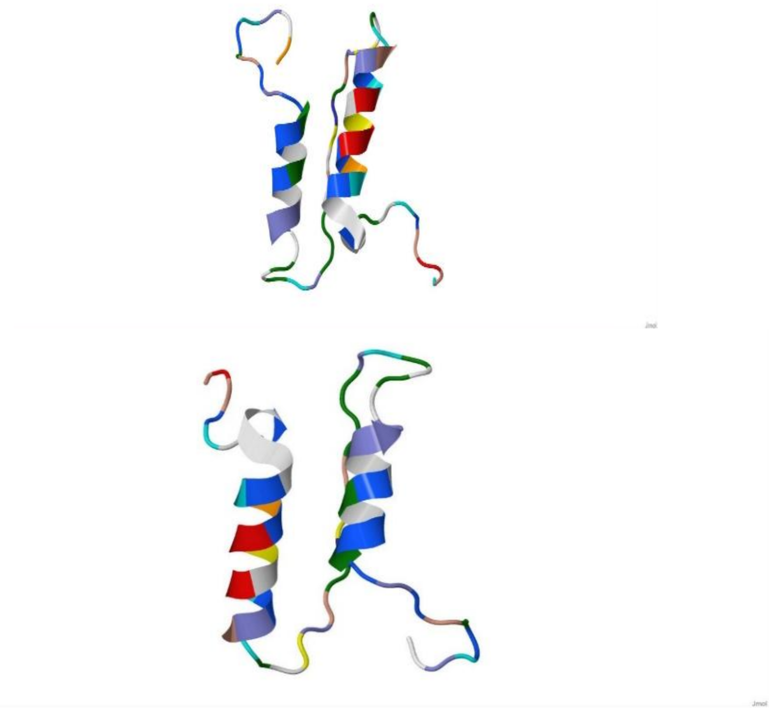


Fig. 4. 3D structure of the ghrelin polymorphism protein in goats.

DISCUSSION

The findings of the current study provide evidence that the GHSR gene in Iraqi local goats contains novel polymorphisms that were not previously recorded in global databases. This supports the concept that local breeds may carry unique genetic variation shaped by local environmental and management conditions, and such variation can be valuable for genetic improvement programs. This considered a genetic diversity that may contribute to animal selection programs based on molecular markers (Habib etal., 2022; Samuel et al., 2022). On the other hand, the presence of two genetic variants in the studied animals may reflect population-level diversity within Iraqi goats. Such diversity can be explained by the genetic background of local herds and may be influenced by adaptation to nutritional fluctuations and environmental stress. In addition, the repeated occurrence of mutations in both variants suggests that some sites may represent shared inherited variations within this population.

On the other hand, studies have indicated that the polymorphism of genes associated with different traits (e.g., productivity) is linked to the possibility of selecting animals with better productivity traits (El-Komy et al., 2020), especially genes that may be molecular markers for selection for specific traits (Rossetti et al., 2023). It is important to note that the similarity of 99.45% with the reference sequence (XM_005675309) indicates close relatedness, yet the lack of complete identity suggests the presence of lineage-specific mutations. These mutations, particularly those repeated in both polymorphisms (21, 22, 1546, 1547, 1548, and 2127), may represent conserved polymorphic sites within Iraqi goats or mutation hotspots in the coding region. Such repeated sites may also support the assumption of a common origin among the studied animals.

In general, none of these mutations can be neglected, as they occurred in an important part of the gene (CDS) that is involved in protein production. On the other hand, silent mutations affect the speed and regulation of protein transcription (Javed et al., 2022), in addition to affecting the mRNA splicing mechanism and thus can directly affect gene expression (Seton-Rogers 2022), while studies have indicated that missense mutations (resulting from an amino acid change) can significantly affect the functioning of the produced protein depending on the differences between the old and new amino acids (Chen et al, 2022). These mutations can enhance the functioning of the protein and thus may represent a molecular marker of selection (Dou et al., 2023). On the other hand, GHSR is a key receptor in ghrelin signaling, which is associated with appetite regulation, growth hormone release, and energy balance. Therefore, variation within the coding sequence may contribute to differences in feed intake behavior and nutrient utilization efficiency among animals. This aspect is particularly important in local goats that may face fluctuating feed availability and environmental stress, where metabolic adaptation could influence growth and productivity. In addition, the predicted effect on the three-dimensional protein structure suggests that some missense substitutions may influence receptor folding and stability. Since GPCR function depends on proper conformation, even minor structural alterations may affect receptor activity and downstream signaling, which could be reflected in animal performance under specific conditions. From an applied perspective, these novel variants may serve as candidate molecular markers for future selection programs in Iraqi goats. However, to confirm their usefulness in marker-assisted selection, association studies are required to link each polymorphism with measurable traits such as growth rate, feed efficiency, and productivity parameters.

It is important to note that the present study was conducted on a limited number of animals and focused on males only, and no direct association analysis was performed between the detected polymorphisms and phenotypic traits. Therefore, further studies on larger populations, including both sexes, with detailed productive and physiological measurements, in addition to gene expression and functional validation, are recommended to clarify the biological significance of these variants.

On the other hand, the results of the study indicate the presence of a number of mutations (whether silent or notice missense) in different locations that were repeated in both genetic formations (21, 22, 1546, 1547, 1548, and 2127), which indicates that these animals are likely of common origin (Hoelzel and Lynch 2023).

Silent and missense mutations can also affect the expected three-dimensional shape of the resulting protein as well as the folding process (Fig. 3). Protein function is directly related to its shape (Zhang et al., 2022). The folding process is also very important as it also affects protein function and resistance to various stress conditions (Durosaro et al., 2023).

CONCLUSION

The results of this study indicate the potential for multiple genetic variations of the ghrelin receptor gene in Iraqi local goats. Furthermore, the mutations identified are novel and have not been previously reported. This suggests the presence of genetic diversity that could contribute to the use of this gene as a molecular marker in genetic improvement and selection programs. Therefore, further studies should be conducted to investigate various productive and physiological traits, such as milk production, feed efficiency, and growth rate, and their relationship to the identified genetic variations of the gene. Additionally, the genetic influence of different mechanisms can be estimated, and correlation analysis between phenotype and genotype can be performed to identify the most distinctive genotypes and thus their potential use in genetic improvement programs.

This study contributes to supporting genetic improvement strategies by identifying desirable genetic mechanisms, which can increase the productive efficiency of Iraqi local goats.

Table 1.

Genetic mutations in the GHSR gene in local male goats

Ghrelin	N.	Reference Gene XM_005675309	Polymorphism	P.	Mutations: Type	Mutations: change
Polymorphism A (LC 8 1 0 4 2 0)	1	T	A	12	Silent	
	2	G	C	21	Silent	
	3	C	A	22	Missense	Proline to Threonine
	4	A	T	300	Missense	Glutamic acid to Aspartic acid
	5	C	T	1021	Silent	
	6	G	T	1022	Missense	Arginine to Leucine
	7	G	C	1546	Silent	
	8	A	C	1547	Missense	Aspartic acid to Proline
	9	T	A	1548	Missense	Aspartic acid to Proline
	10	T	C	1565	Missense	Phenylalanine to Serine
	11	C	G	1882	Missense	Proline to Alanine
	12	G	A	2127	Silent	
	13	A	C	2162	Missense	Glutamic acid to Alanine
Polymorphism B (LC 8 1 0 4 2 1)	1	G	C	21	Silent	
	2	C	A	22	Missense	Proline to Threonine
	3	C	A	590	Missense	Alanine to Aspartic acid
	4	A	T	716	Missense	Glutamine to Leucine
	5	C	G	1448	Missense	Alanine to Glycine
	6	G	C	1546	Silent	
	7	A	C	1547	Missense	Aspartic acid to Proline
	8	T	A	1548	Missense	Aspartic acid to Proline
	9	A	G	1713	Silent	
	10	G	A	2127	Silent	
	11	T	C	2207	Missense	Phenylalanine to Serine
	12	C	T	2208	Missense	Phenylalanine to Leucine
	13	T	C	2209	Silent	
	14	A	G	2235	Silent	
	15	A	C	2346	Missense	Lysine to Asparagine

AUTHORS CONTRIBUTIONS

M.K.M.: Collecting samples and conducting laboratory work, as well as writing the initial draft of the manuscript.

H.N.H. and W.Y.K.: Supervising the research, designing the study, reviewing the research scientifically, contributing to the methodological aspects, analyzing the results, and reviewing the research.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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