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Genetic Analysis of Growth Hormone (GH1) Gene in Three Indigenous Goat Breeds of District Larkana, Sindh, Pakistan

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ABSTRACT

Pituitary growth-promoting cells, located near the base of the brain, generate growth hormone protein, which is encoded by the GH1 gene. Bone and tissue development is dependent on growth hormone, which is produced by the pituitary gland. Growth hormone is inhibited or hindered by GH1 gene mutations. Insufficient levels of growth hormone cause the body to grow more slowly, resulting in short stature. Using DNA sequencing and PCR gel electrophoresis, researchers found six GH-1 gene variants in each of three goat breeds: Sindh Desi goat breed (03), Tapri goat breed (02), and Kamori goat breeds (03). (01). Point mutations in the genetic code are those that affect only one gene, such as mutation 03 in Sindhi goats, mutation 02 in Tapri goats, and mutation 01 in Kamori goats. According to this research, the Sindhi goat's meat qualities are enhanced by the breed's unique DNA sequence.

Keywords: DNA quantification, Goat breeds, PCR, DNA sequencing

Introduction

Growth is a multi-gene, environment-controlled trait. Growth hormone (GH) and insulin-like growth factor-I (IGF-I), which comprise the hypothalamus' base, are the most important genes. (Hormone, Pituitary, Growth Promoting) Axis of the This biological process is governed by a complex network of genes, hormones, and environmental factors. There are several hormones produced by the pituitary gland, the most well-known being growth hormone. The GH1 gene in humans produces this protein. Biochemically speaking, growth hormone is a polypeptide hormone produced by the pituitary gland. Body composition, appetite control, and reproduction are all impacted by growth hormone. Growth hormone is also involved in egg laying and growth. All three regions of the bovine genome's introns had growth hormone polymorphisms. A connection has been found between the cow's GH gene and milk protein content, according to the milk's protein content. Putnova et al. (1999) and Vasilators-Younken et al. (1999) are examples of studies that have examined the effects of dietary fibre on blood pressure and cholesterol levels in the body.

The GH gene's intron and exon regions have been shown to influence goat meat quality. The glucocorticoid regulatory element, located in the first intron of the HGH gene, may have a role in the transcriptional control of human GH (HGH). Moore and colleagues (1985; Slater and colleagues (1985) are examples of this; The demand for sheep has resulted in a significant shift in sheep breeding, and high-quality growth is necessary to enhance ewe weight and size. Growth hormone,

also known as anabolic hormone, is created and released by growth hormone cells in the pituitary's frontal lobe in response to the circadian rhythm and pulse pattern (Ayuk and Sheppard, 2006).

Fast-maturing lambs attain market weight at a younger age as a result of breakthroughs in biotechnology and biology, which means they require less feeding time, a lower risk of death, more precise selection, and better organization. This goal can be achieved with the help of marker assisted selecting (MAS). In wool production, using genetic makeup can assist identify animal potential earlier and enhance selection accuracy and efficiency. Growth hormone (GH) genes have been linked to dietary quantity and quality in numerous studies. The GH gene is important for sheep development and management. 2007 (Itenge-Mweza and colleagues).

The rate at which you grow is greatly influenced by the GH gene. Bauman is a novelist and journalist based in New York City (, 1989). Breir (1999), Bald (1999), Akers (2006), Scaramuzziet et al. (1999) and metabolism (Breir and colleagues, 1999) all play an essential role in farm animals' growth and development (Bauman, 1999). GH genes' capacity to identify patterns is critical (polymorphism). The GH gene variant had a role in four goat tribes in China, according to the study (An et al. 2010). The Zaibu (Bos indicus) cow breed in India has a GH gene polymorphism (variation) that researchers identified (Sodhi. Et, al 2007). GH gene polymorphisms were discovered in Japanese black cattle, Holstein cows, Herford cattle, and Aberdeen-Angus cattle (Chikuni. et al. 1994). While the GH gene is used to detect genetic alterations in individuals all over the world, it is not utilised in cattle (Maheshwari. et al. 1998). Study looked at goat breeds' genetics for the GH gene due to its significance because of the GH gene.

Material and Method:

1. Blood Sample Collection

A total of 30 blood samples were taken from three goat breeds: Kamori goats, Sindh Desi goats, and Barbari goats. Each DNA sample will be used to investigate (03) the allele/genotype frequency of the GH gene in various cattle breeds in order to compile a polymorphism database. The animals were chosen using the Canadian Animal Health Commission's standards (MacNeil and Newman, 1994).

ii DNA Extraction

DNA will be extracted using Thermo Scientific Gene Jet # K0781 Whole Blood Genomic DNA Extraction Kit and stored in a refrigerator at -20oC (Kruger et al., 2017).

Detailed methodology is given below

Steps:

1. To obtain a homogenous suspension, add 20 µlitres of proteinase K solution to 200 µ
litres of whole blood, mix by pouring, and then add 400 µlitres of lysate by pouring or pipetting.
2. Incubate the sample for 10 minutes at 56°C in a shaking water bath until the cells are totally lysed.
3. Using a pipette, mix 200 l of ethanol (96-100 l).
4. The resultant mixture was then put in a spin column and centrifuged at 6,000 x g for one minute (- 8,000 rpm).
5. To this add 500 mL of WB 1 washed with ethanol and centrifuge for one minute at an acceleration of eight thousand times g (- 1000 amperes).
6. Fill the column halfway with Wash Buffer II and let it sit for 30 minutes (with ethanol). Centrifuge at maximum speed for 3 minutes (14,000 rpm x 20,000 g).
7. Finally, add 30 L of elution buffer to the column membrane and incubate for 2 minutes to elute the genomic DNA. Centrifuge for one minute at 8,000 x g (around 10,000 rpm) at room temperature.

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8. Purified DNA can be used right away or kept indefinitely at 20°C for future use when the other steps have been completed.

iii. Quantification of DNA

The quality of DNA was checked on 1.5% agarose gel by OD method using a nano dropper (Desjardins and Conklin, 2010).

iv. Primer Design and PCR Amplification

The first was created with the original Premier 3 software and synthesized by Penicon. In a 29L container, PCR amplification was carried out according to normal procedures (Zhang et al., 2007). For every 50 mg template DNA, 10 pM of primers, and 2.5 mg of MgCl2 in the reaction mixture, add 50 mg of each. Then add the 50 mg of primers, 10 pM of dNTPs, 2.5 mM of MgCl2, and 0.5 U of DNA polymerase to the reaction mixture (Jiang et al., 2014). The following conditions will be met throughout the PCR process. There will be 32 cycles of denaturation at 940°C for 5 minutes, then 3 seconds of denaturation. 30 seconds at 580 degrees, 30 seconds at 72 degrees, 30 seconds at 72 degrees, and 10 minutes at 72 degrees. Use 1X TBE buffer (89 mM Tris 89 mM, boric acid, 2 mM Na2EDTA) on a 1.5 agarose gel (stained with 200 mg/ml ethidium bromide) to electrophorese PCR findings. Table 1 summarizes all of the primers' information.

Table 1 Primers used for PCR amplification and their details

Primers	Forward 5` - 3`	Reverse 5` - 3`	bp	Tm
GH1 gene	ATAGGGGAGGGTGGAAAATG	TACGTCTCCGTCTTGTGCAG	210	58
	ATAGGGGAGGGTGGAAAATG	AAGCAGGAGAGCAGACCA	210	58

v. PCR production Purification and Sequencing

The PCR products were commercially purified and sequenced from Macrogen Korea (https://dna.macrogen.com/).

Data Analysis

The data was analyzed by comparing the obtained sequence with the known sequence in the NCBI database. (http://www.ncbi.nlm.nih.gov) In addition, an Excel computer program is used to calculate the number and percentage of mutations.

Results

Quantified DNA

The amount of DNA in the extracted DNA sample was measured using a nanodroplet spectrophotometer, and the gel documentation system was determined to be in the range of 30-103 ng/L, which was suitable for PCR amplification. In Figure 1, the findings of DNA quantification are shown.

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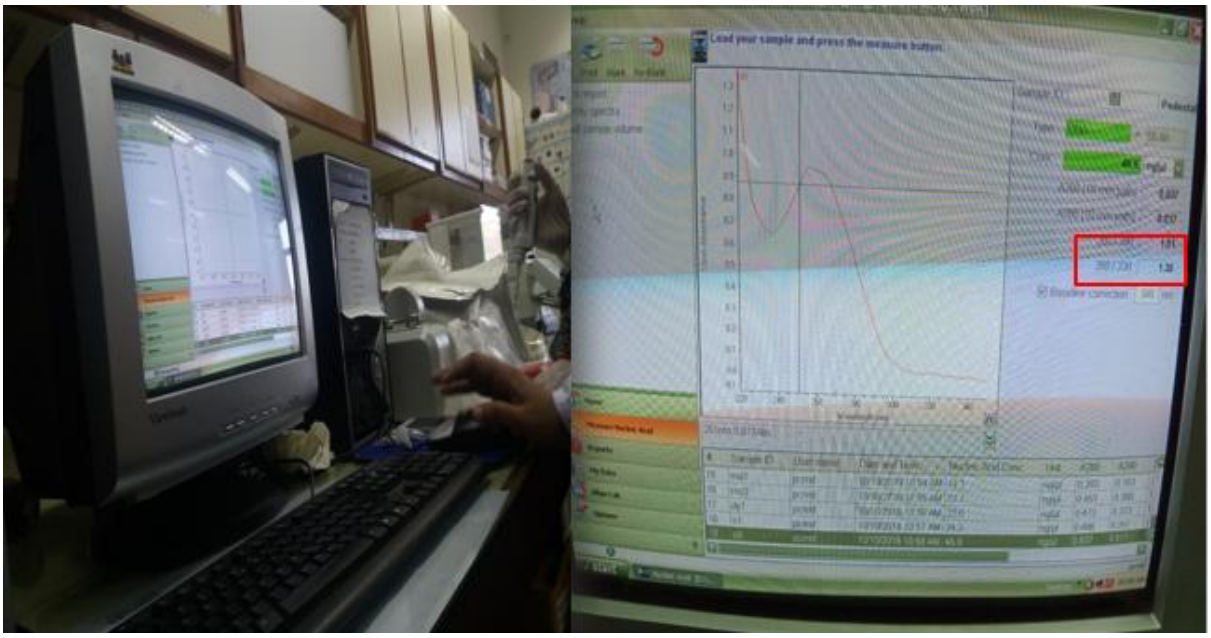
Graph 1: Quantification of DNA using the Nanodrop Technique.



Polymorphism identification based on Genetic code of PCR product of Gh gene

Gh gene was PCR amplified in all three breeds, and size was verified (210bp) as shown in Fig 1 and SNPs were identified. Following PCR amplification, the amplified product was sequenced. The resulting sequence for the Gh gene FASTA sequence (is shown as supplementary data in the end). The sequence alignment tool ensemble.org was used to evaluate the sequence online. The alignment graph depicts the following mutations following the genetic code.

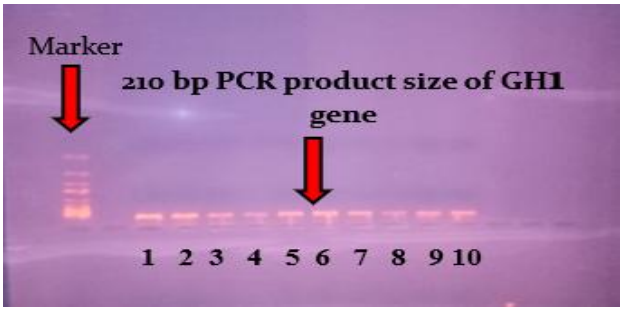


Fig 1 PCR amplified Product of Gh gene

GH-1 gene variations were found in three goat breeds: the Sindh Desi (03), Tapri (02), and Kamori (03) varieties (03). (01). This study found that the GH1 gene in the three goat breeds had a total of six single nucleotide variants. All three Sindhi goat breeds in the Rakana region of Pakistan's Sindh Province carry the GH1 gene. Three SNPs were found in the Sindhi goat breed, one in the Kamori goat breed, and two in the Tapri goat breed using PCR-based study. The genetic code classifies the aforementioned alterations as point mutations; more information may be found in Tables 2 and 3.

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Table 2 detailed description of mutations in the Gh gene

Sample	Position of mutated NB	Original codon	Changed codon	Original A. A	Changed A. A
Sindhi Desi	229	AAT	AAG	Aspartic acid (non-essential)	Lysine (Essential)
	29	TCC	TCG	Serine (Non-essential)	Serine (Non-essential)
	23	TAT	TCT	Tyrosine (Non-essential)	Serine (Non-essential)
Kamori	09	GTC	GTG	Valine (Essential)	Valine (Essential)
Tapri	47	CTC	TTC	Leucine (Essential)	Phenyl alanine (Essential)
	37	CAG	CAC	Glutamine (Non-essential)	Histidine (Essential)

Table 3 Counting Percentage of mutations in three breeds of cattle

Gene	Name of Breed	Found SNPs	Method	Total
GH-1	Sindh Desi	3	3/210×100/=	1.42%
	Tapri	2	2/210×100/=	0.95%
	Kamori	1	1/210×100/=	0.47%

Note: Polymorphism arises when the population's mutation rate is larger than one. Simply put, given the above-mentioned population genetic criteria, Sindhi Desi goat breeds display polymorphisms, whereas other breeds only show mutations.

Discussion

It's been shown that among these three goat breeds, the GH1 gene contains 06 different single nucleotide alterations. All three Sindhi goat breeds in the Rakana region of Pakistan's Sindh Province carry the GH1 gene. Three SNPs were found in the Sindhi goat breed, one in the Kamori goat breed, and two in the Tapri goat breed using PCR-based study. Point mutations in the genetic code are what are happening here.

The mutation occurred at base pair 229 of the Sindhi goat breed when aspartic acid was changed to lysine. The non-essential amino acid aspartic acid's original codon AAT is transformed to the necessary amino acid lysine's AAG, and the mutation is meaningless. Another mutation occurs when cytosine is changed to guanine at nucleotide 29, resulting in the conversion of the previously translated glycine codon, TCC, to TCG, which encodes the same amino acid.

A single mutation in the Sindhi goat breed transforms adenine to cytosine at base pair 23. TAT, which codes for the amino acid tyrosine, was wrongly converted to TCT, which codes for the amino acid serine. There are two varieties of the Kamori goat race's sixth base pair. GTC used to stand for leucine, but it was changed to GTG and currently stands for valine. The Tapri goat breed has two SNPs, one at nucleotide 47 and the other at nucleotide 37. The prior CTC base pair encoding leucine has been changed to phenylalanine, a necessary amino acid for TTC. CAG, which originally designated for the amino acid glutamic acid, has been changed to CAC, which now codes for the amino acid histidine. The new study's findings are in line with Ujjan et alwork .'s from 2011.

Conclusion

Six GH-1 gene variants were found when DNA sequencing and PCR gel electrophoresis were used to examine three goat breeds: the Sindh Desi goat breed (03), the Tapri goat breed (02), and the goat breed Kamori (03). (01). Point mutations are what the genetic code calls these kinds of changes. Sindh Desi goats have a 3-point mutation, Tapri goats have a 2-point mutation, and Kamori goats have a 1-point mutation. Our findings show that the Sindh Desi goat breed may have contributed to the meat's flavour and texture due to substantial variations in its DNA sequence.

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