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GENETIC CHARACTERIZATION OF THE GROWTH HORMONE GENE IN CAMEL (*Camelus dromedarius*) POPULATIONS IN NIGERIA

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Abstract

This study characterized the growth hormone (GH) gene in Nigerian camel populations sampled from Zamfara and Katsina states, using molecular techniques. Blood samples were aseptically collected randomly selected camel population in Zamfara and Katsina States, Nigeria. Genomic DNA was extracted from the blood samples collected and Polymerase Chain Reaction (PCR), using primers KGH3-F and KGH3-R, was carried. A 687-bp fragment of the camel Growth Hormone (GH) gene extracted was evaluated via gel electrophoresis. Nucleotide sequence obtained from sequencing, were analyzed, using Bioinformatics comparison (NCBI), revealed 99.67% similarity between Nigerian *Camelus dromedarius* GH sequences and the reference sequence AJ575419 from *Camelus dromedarius* in the United Kingdom. BLAST alignment of the sequenced fragments against NCBI references revealed 99.67% similarity and 99% query coverage with the reference GH sequence from a UK-derived *Camelus dromedarius* (AJ575419). The alignment overlapped intron 4 and exon 5 of the gene, with two minor gaps observed at positions 6 and 15. These indels, representing only 0% of the sequence, may reflect either natural polymorphisms or minor sequencing artifacts. No single nucleotide polymorphisms (SNPs) were detected in the sampled population, suggesting low genetic variability and a common ancestry among Nigerian camels. The findings suggest high conservation of the GH gene across geographically distinct camel populations, highlighting its potential role in adaptation and productivity. The study establishes baseline genetic data for Nigerian camels, with implications for improving camel productivity and informing breeding programs in arid regions. It however, highlights the need for further studies with larger sample sizes to assess the relationship between GH gene variations and phenotypic traits such as growth rate and milk yield for targeted breeding programs in arid regions.

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INTRODUCTION

Camels (*Camelus dromedarius*) are vital to livelihoods in arid regions like Northwest Nigeria, contributing to milk, meat, and transportation, this is due to their drought resilience and economic value (Rotimi *et al*., 2023).

The growth hormone gene (GH) is known to significantly influence key production traits, such as growth rate, muscle mass, and milk yield in animals. Growth hormone gene has proven to be the major regulator of postnatal growth and metabolism in mammals including camels and thus affects growth rate, body composition, health, milk production and aging by modulating (regulating) the expression of many genes (Mohammad *et al*., 2021; Carnicella *et al*., 2003). In livestock, growth hormone genes are of economic importance because they are often associated with faster growth and reduced fat stores (McMahon *et al*., 2001). Investigating the GH gene in Nigerian camels is expected to reveal genetic variations that can be harnessed for breeding programs designed to enhance these traits.

Research in other livestock species has demonstrated that genetic screening for the GH gene can effectively identify individuals with superior growth potential (Zhou *et al.,* 2014). This knowledge is essential for developing targeted breeding strategies that prioritize productivity and adaptability. A preliminary screening of the GH gene could yield valuable insights into the genetic profile of these camels, facilitating selective breeding and

ultimately improving their productivity (Bertolini *et al.,* 2018).

The growth hormone gene regulates key physiological traits, including growth, metabolism, and lactation (Mohammad *et al*., 2021). The growth hormone, known as somatotropin, is a protein hormone made up of roughly 190 amino acids. It is synthesized and secreted by somatotroph cells in the anterior pituitary gland. This hormone plays a crucial role in regulating various physiological processes, such as growth, metabolism, lactation, and the development of mammary glands in animals. The camel growth hormone (GH) gene is approximately 1900 base pairs in length and, similar to other mammalian GH genes, contains five exons and four introns (Ishag *et al*., 2010). Despite its importance, genetic studies on Nigerian camels are scarce, and existing research focuses on populations outside Africa (Maniou *et al*., 2004). Most research on candidate genes has focused on cattle (Ishag, 2009), sheep (Bastos *et al.,* 2001), and goats (Rotimi *et al.,* 2020), while studies on the growth hormone gene in camels remain limited. This trend leaves gaps in understanding local genetic diversity. This information gap limits efforts to optimize camel productivity through genetic selection.

There is insufficient molecular data on the genetic diversity of growth hormone genes in Nigerian camel populations. This hinders evidence-based breeding programs and understanding of camel adaptation to local environmental stressors, especially in regions like Zamfara and Katsina, where camels are economically significant.

This study addresses the gap in genetic characterization of Nigerian camels, providing actionable insights for agricultural and biotechnological applications. The GH genes role in growth and lactation makes it a strategic target for improving camel-derived resources in arid economies. Therefore, the objectives of this study were to amplify and sequence the GH gene region in Nigerian camels and to compare with global sequence references (NCBI).

MATERIALS AND METHODS

Study location

The study was conducted in two states located in the northern region of Nigeria: Katsina and Zamfara. These states were purposively selected due to the significant population of camels in the area. Consequently, three Local Government Areas (LGAs) were chosen for the study: Mai-Adua and Charanchi LGAs in Katsina State, and Gusau LGA in Zamfara State. These LGAs were selected based on their relative security levels. Mai-Adua is found within latitude of 13.1799° N and a longitude of 8.2304° E, Charanchi within latitude of 12.6716° N and a longitude of 7.7293° E, while Gusau is located at a latitude of 12.1702° N and a longitude of 6.6641° E (Geodatos.net).

Experimental animals and their management

A total of fifty-one (51) camels, consisting of 24 females and 27 males, were randomly sampled from the three selected Local Government Areas in Katsina and Zamfara States. The camels were randomly selected from various locations within the study area. Only healthy, unrelated, and non-pregnant camels were included in this study.

Data collection

Blood Samples Collection

About 2 mL of whole blood samples were aseptically collected from the jugular veins of healthy individual camels. The samples were preserved in blood collection tubes containing EDTA as an anticoagulant. To prevent spoilage during collection and transportation, the blood samples were stored in an ice pack, transported to the laboratory, at -4°C until nucleic acid extraction.

Genomic DNA Extraction Procedure

Genomic DNA was extracted from whole blood samples using the phenol-chloroform extraction method described by Sambrook and Russell (2006). Initially, blood samples were thawed at room temperature.

Tris-EDTA lysis buffer was added to the thawed samples, followed by the addition of approximately 20 mg/mL of proteinase K. The mixture was thoroughly mixed and incubated at 55°C for 30 minutes to 1 hour.

Next, an equal volume of phenol-chloroform was added to the sample and mixed thoroughly. The mixture was then centrifuged at 10,000 x g. The aqueous phase was carefully collected and transferred to a new tube. To the aqueous phase, 2-3 volumes of ice-cold 95% ethanol were added, and the mixture was thoroughly mixed before incubating at -20°C. The sample was subsequently centrifuged at 10,000 x g for 10-15 minutes. The supernatant was carefully discarded, leaving the DNA pellet behind. The DNA pellet was washed

with 70% ethanol and then re-suspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). Finally, the extracted DNA was stored at -20°C.

Polymerase Chain Reaction (PCR) and gel electrophoresis

Polymerase Chain Reaction (PCR) was performed using a pair of primers of the Camel Growth Hormone (GH) gene based on published nucleotide sequence information (GenBank accession no. AJ575419, Maniou *et al*., 2004). The primer sequences are presented in Table 1. High annealing specificity (57°C) and GC content (57.89%) contributed to successful amplification. The resulting fragments were visualized using chromatogram analysis to determine the precise genetic sequence of the DNA samples.

Gel Electrophoresis

The quality of the extracted DNA samples was assessed using 1.5% agarose gel electrophoresis. DNA samples, dyed with ethidium bromide, were loaded onto the gel alongside a standard gene ladder. The contents were subjected to electrophoresis at a specified voltage for about one hour. DNA bands were visualized using a UV light box.

DNA Sequencing

The purified extracted fragments were sequenced using a BigDye Terminator v1.1 Ready Reaction Cycle Sequencing kit (Muhammad *et al*., 2021) and an ABI Prism 310 Genetic Analyzer (Applied Biosystems, USA). The results were submitted for alignment on NCBI website. The sequences were aligned and the presence and position of SNPs were evaluated with reference sequence of accession number AJ575419 as reported by Maniou *et al.* (2004). This work was carried out at the DNA Laboratory, Kaduna, Kaduna state, Nigeria.

Data analysis

The basic information on the gene sequences obtained was analyzed using Basic Local Alignment Search Tool (BLAST) procedures. This tool compares nucleotide sequences against sequence databases maintained by the National Center for Biotechnology Information (NCBI), facilitating the identification of genetic similarities and variations.

Table 2: The primer sequences and size (bp) of Camel GH amplified fragments

Name	Sequence (5'-3')	GC Content	Tm	Product size	T_A
KGH3-F	CTTCTCGCTGCTGCTCATC	57.89 %	60 ^{0}C	687 bp	57^{0}C
KGH3-R	GCACTGGAGTGGCACTTTC				

Tm = Melting Temperature, T_A = Annealing Temperature

RESULT AND DISCUSSION

Polymerase Chain Reaction (PCR) Result

Figure 1 shows the PCR gel image obtained through agarose gel electrophoresis. Gele electrophoresis is a fundamental technique in molecular biology used to separate DNA fragments based on their size. A molecular marker of 100 bp size was employed during gel electrophoresis in this study, as a reference for estimating the size of the PCR products.

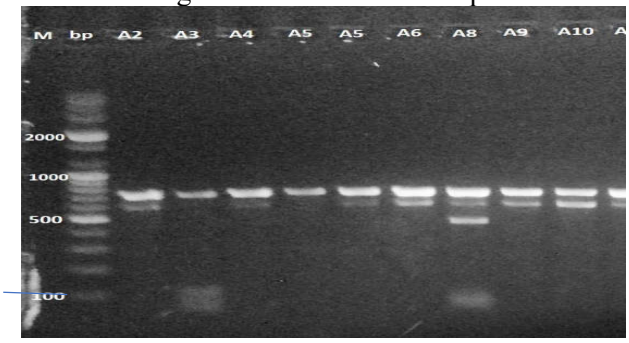

Plate 1: Electrophoretic image of the amplified region of the camel growth hormone gene.

The bands at the expected sizes confirm the correct amplification of the target DNA sequence. A 687-bp GH fragment was successfully amplified (Plate 1, Table 3). This is close to the report of Ishag (2009), who amplified a 613-bp fragment that encompasses the mutation site containing single nucleotide polymorphisms (SNPs) in the camel growth hormone (cGH) gene of Sudan population.

Sequence Result

Nucleotide sequencing was performed using the dideoxy nucleotide chain-termination technique of Sanger *et al.* (1977). The DNA sequence results from the samples are presented in chromatogram format (Plate 2).

Table 3 presents detailed basic information of the amplified region of the camel growth hormone (cGH) gene. This amplified region was submitted to the National Center for Biotechnology Information (NCBI) website to

access fundamental information regarding the amplified gene region. The Basic Local Alignment Search Tool (BLAST) procedures was employed to identify regions of similarity between the sequences and the various sequences submitted to the NCBI. The result of the BLAST is presented in Table 3. The results revealed that the size of the amplified gene was 687 bp. More than one hundred sequences from other species were found to be similar to this sequence, with similarity percentages ranging from 86.51% to 99.67% (Table 3). The nucleotide information aligns with the published nucleotide sequence of the camel GH gene (GenBank accession no. AJ575419) as reported by Maniou *et al.* (2004).

The second sequence in the search table, with Accession No. AJ575419.1, was employed as the reference sequence. A query coverage of 99.00% was obtained for this search. Querry coverage is used to evaluate the extent of similarity between a query sequence and sequences in a database. The result shows a high degree of similarity which implies that the query sequence and the reference sequence selected are highly identical. An expected value (E-value) of 0.00 was also obtained in the query search. This indicates a more significant matches in the search result. The nucleotide information aligns with the published nucleotide sequence of the camel GH gene (GenBank accession no. AJ575419) as submitted by Maniou *et al.* (2004). 99.67% sequence similarity to AJ575419 (UK-derived GH sequence) suggests high evolutionary conservation. Minor variations may reflect regional adaptations or sequencing artifacts.

The results of the alignment also reveals that the alignment between the amplified sequence and the query sequence (Accession No. AJ575419.1) coincides with intron 4 and exon 5 of the queried sequence, covering approximately 687 bp. The alignment results of the submitted sequence with the GenBank sequence (AJ575419) derived from dromedary camels, show two gaps at positions 6 and 15 in the sequences. In BLAST alignment, gap represents an insertion or deletion (indel) of one or more nucleotides in either the query or subject sequence. Two gaps were observed in this alignment, 2 out of 601 nucleotides (0%). Since the alignment spans exons 1–5 of the growth hormone (GH) gene, gaps in coding regions can be significant. The two gaps, presents possible frameshift mutation, as they occur within a coding exon. But given the 99% identity, this may be a sequencing error, natural polymorphism, or species-specific variation (Primus, 2010). However, the two gaps might represent small evolutionary changes between the query and subject sequences.

The absence of SNPs in the GH gene of the sampled camels suggests that these ecotypes originated from a common or closely related stock, and not enough time has elapsed for segregation and the emergence of new mutants (Ishag, 2009).

Ishag (2009) observed one SNP in Sudanese camels located in a non-coding region (intron 1) at position 419C>T. Shah (2006) also reported the presence of SNPs in Pakistani dromedary camels. Numerous authors have documented various mutations (manifestations of SNPs) in Nigerian goat breeds (Rotimi *et al*., 2020), and SNPs in Black Bengal goats (Gupta *et al.,* 2007) and sheep (Marques *et al.,* 2006).

Presence of SNPs in gene sequence possess potential significance on various aspects such as growth rate, reproduction, milk production.

Table 3: Basic information of the Nucleotide Sequence of the camel GH gene region amplified in NCBI search

Isolate	Closest ancestor	Similarity	Query coverage	Query length	E value	Accession No	Reference
Camel blood sample	*Camelus dromedarius* growth hormone, exons 1-5 Location – United Kingdom	99.67%	99%	687bp	0.00	AJ575419	Maniou *et al.* (2004)

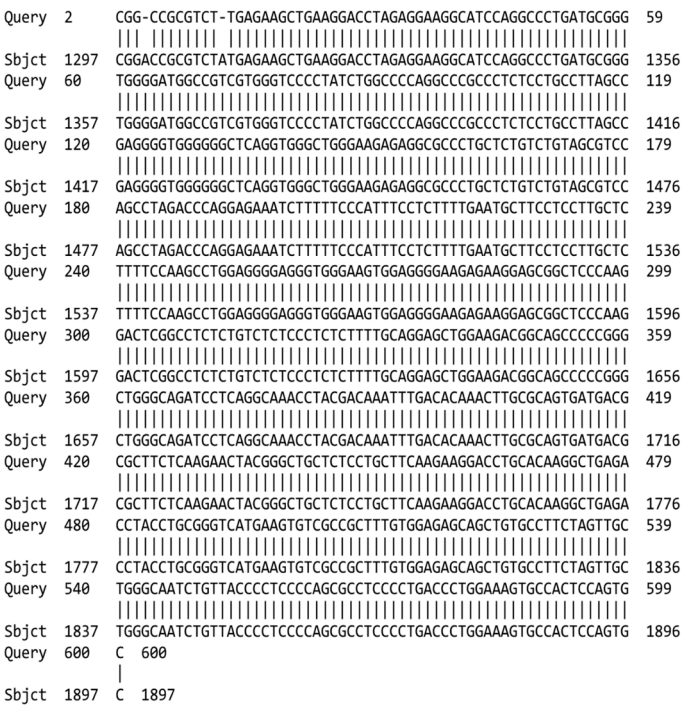

Plate 2: Nenucleotide alignment with NCBI database

CONCLUSION

The results revealed that the GH gene in Nigerian camels is highly conserved, with near-identical sequences to global counterparts. This shows a remarkable genetic conservation with global populations. This stability may reflect demographic confinement. Further study involving expanded sample size is recommended to investigate the associations between GH gene variants and phenotypic traits such as milk yield.

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