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DETECTION OF POLYMORPHISM IN EXON-3 REGION OF LEPTIN GENE IN BIKANERI CAMEL

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ABSTRACT

The study was undertaken on 40 Camels (*Camelus dromedarius*) of Bikaneri breed to identify the single nucleotide polymorphism (SNP) in leptin gene. Nucleotide sequence of *Lama glama* was used to design primers to amplify selected region of leptin gene in Bikaneri camel. A 540bp fragment was amplified which covered Partial intron-2, exon-3 and partial 3′ UTR region of leptin gene in Bikaneri camel. The genetic variation in partial ntron-2, exon-3 and partial 3′ UTR (540bp) region of leptin gene was studied utilising single strand conformational polymorphism (SSCP) technique followed by nucleotide sequencing. SSCP analysis revealed monomorphic pattern as only AA genotype in studied region of leptin gene was observed. For verification and validation of observed results of SSCP, amplified PCR products of 6 animals were randomly selected for sequence analysis to ascertain the sequence pattern and existence of point mutations in the selected region. All the 6 animals had same sequence pattern. Monomorphic pattern showed the conserved nature of leptin gene in dromedary camel.

Key words: Bikaneri camel, exon-3, Leptin gene, *Lama glama*, polymorphism

Leptin, the product of the LEP gene, is a 16-kDa protein synthesised by adipose tissue and is involved in the regulation of feed intake, energy balance, growth, fertility, production traits and immune functions. Delavaud *et al* (2013) observed vital role of plasma leptin in adaptation of camel in harsh environmental conditions such as food and water shortages. Leptin and camel milk plays a significant role to abate oxidative stress (Hamzawy *et al,* 2018). PCR based single strand conformational polymorphism (SSCP) is more useful and easy technique to detect single nucleotide polymorphism (SNP) in large population. SSCP analysis has benefits over restriction fragment length polymorphism (RFLP) due to revealing more polymorphisms at various positions in DNA fragments (Orita *et al,* 1989). SSCP detects single nucleotide polymorphism by uncharacteristic electrophoretic passage of one or both single strands in a non-denaturing polyacrylamide gel (Gruszczynska *et al,* 2005). SNPs detection using SSCP is appropriate marker for the removal of sequencing errors during high density mapping (Vignal *et al,* 2002).

The leptin gene is comprised of 3 exons and 2 introns of which only exon 2 and 3 are translated into protein (Bruce *et al,* 1997). The sequence pattern of the leptin gene is known for many livestock species. However, very few entries are available for the camel leptin gene in the NCBI nucleotide database repository so designing of primers were done using nucleotide sequence of leptin gene in *Lama glama*. Considering the limited information available for the camel leptin gene, the objective of this study was to reveal the genetic polymorphism in exon-3 region of leptin (LEP) gene in Bikaneri camel breed of India.

Materials and Methods

Animals and sampling

All animal experiments were performed after approval of Institutional Animal Ethics Committee (IAEC). Forty camels (*Camelus dromedarius*) of Bikaneri breed were selected as representative sample to identify the single nucleotide change in leptin gene. Out of these, 28 were lactating females and 12 were male progenies of selected females. The Bikaneri breed of camels for the trial were selected from ICAR-NRCC Bikaner, Rajasthan, India.

About 10 ml of blood was collected aseptically through jugular vein puncture into anticoagulant

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EDTA containing vacutainer tube from selected animals and stored at 4°C till isolation of genomic DNA.

DNA extraction

Genomic DNA was isolated from the blood samples by 'Phenol Chloroform extraction method (Sambrook and Russel, 2006) with slight modification. The integrity and quality of extracted DNA were checked on 0.8% agarose under horizontal gel electrophoresis and visualised under gel documentation system.

Gene amplification

The leptin gene sequence of *Lama glama* available online in the GenBank (www.ncbi.nlm.nih.gov) (Acc. No KC295539.1) was used for primer design due to unreported genomics sequence in camel. In this study, 540 bp fragment length primer was designed to detect polymorphism which covered partial intron-2, exon-3 and partial 3' UTR region of leptin gene using primer-3 software (Untergasser *et al*, 2012). Forward "TGATGTGGAAGGGAGAAAGG" and Reverse "AGACACCTGGAAGCTCAGGA" primer sequences (5' to 3') were used to amplify the desired region. For amplification, tubes with a final volume of 25 µl containing 2 µl (50ng/µl conc.) of template DNA, 10 mmol/µl conc. of each primer, 13 µl GoTaq® Green Master Mix, 2X (PROMEGA, USA) and 7 µl of nuclease free water were inserted in applied biosystem gradient thermocycler. The amplification program consisted of an initial denaturation at 95°C for 5 min, then 34 cycles of denaturation at 94°C for 1 min, annealing at 56°C for 45 seconds, elongation at 72°C for 45 seconds, and a final extension of 72°C for 10 min. Amplified samples were held at 4°C for 5 min for better stability and condensation.

Analysis of PCR products

The quality and size of the PCR amplicons were assessed on 1.5% agarose gel. The size of the amplified DNA fragments was assessed by comparison with standard molecular weight marker as shown in Fig 1.

Leptin gene polymorphism

PCR based single strand conformational polymorphism (SSCP) technique was used for analysis of leptin gene polymorphisms. Each PCR product was diluted in a denaturing solution (95% formamide, 0.025% xylene cyanol and 0.025% bromophenol blue, 25 mM EDTA), denatured at 98°C for 10 min, immediately chilled on ice and resolved on 10% denaturing urea polyacrylamide gel. The electrophoresis was performed at 4°C temperature for 8h using refrigerator in a vertical mini gel electrophoresis system. The gel were stained with 1% ethidium bromide solution and visualised under UV light gel documentation system. Representative PCR products of electrophoresed SSCP patterns sequenced for both direction in a commercial laboratory (X celris Genomics Pvt. Ltd, Ahmedabad, India) through Sanger dideoxy chain termination method (Sanger *et al*, 1977). The nucleotide sequence was submitted to the GenBank database with accession no. MT103545.1. Elbers *et al* (2019) submitted nucleotide sequence of leptin gene of Arabian camel to the NCBI GenBank database with gene ID 105085360.

Results and Discussion

A 540bp fragment of leptin gene was amplified. PCR-SSCP of the amplified leptin fragment was performed to detect any mutation. Several combinations for concentration of acrylamide:bisacrylamide in urea polyacrylamide gel mix for SSCP were tried to get optimum resolution and migration of the amplified products.

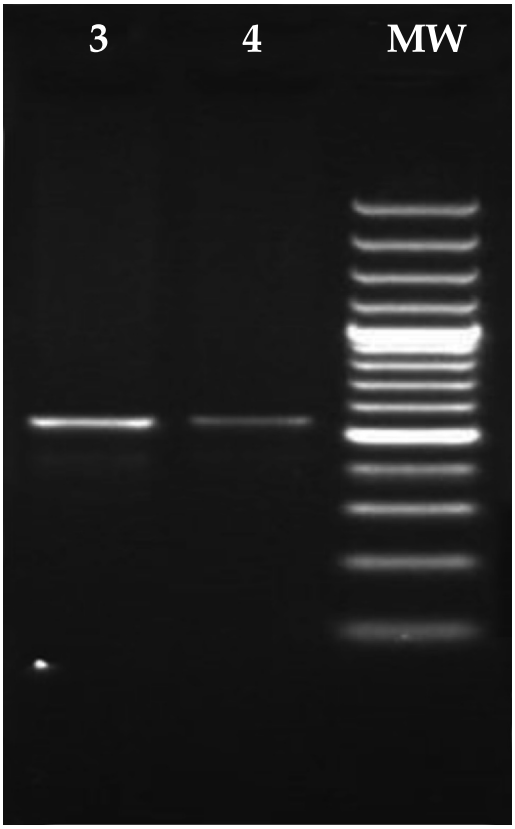

Fig 1. Amplification of camel leptin gene, partial intron-2, exon-3 and partial 3' UTR region (540 bp, lane 3-4), MW: Molecular weight marker (100 bp).

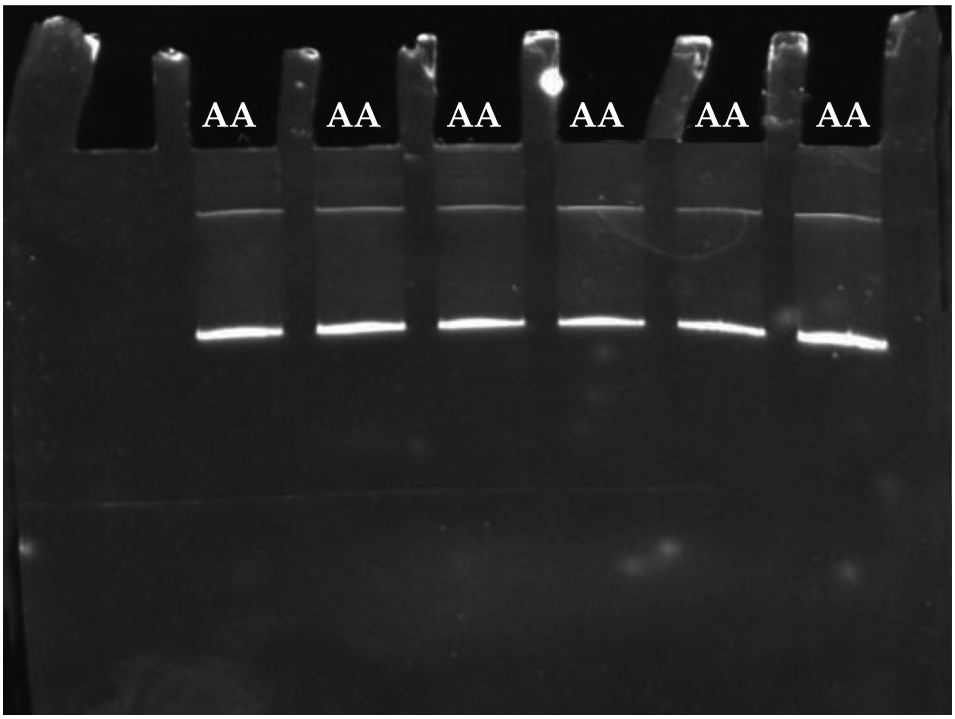

Fig 2. PCR-SSCP for partial intron-2, exon-3 and partial 3′ UTR region (540 bp) of leptin gene in Bikaneri breed camel.

In all 40 Bikaneri camels same band pattern denoted as AA, was observed as shown in Fig 2. However, the polymorphism in the exon 3 of the LEP in sheep was reported by Zhou *et al* (2009) using PCR-SSCP technique. For the verification and validation of observed results of SSCP, amplified PCR products of 6 animals were randomly selected for sequence analysis to ascertain the sequence pattern and existence of point mutations in the selected region. After sequencing in both direction of PCR product of amplified fragment and its editing, a single contiguous of 544bp length was confirmed. In initial sequence analysis, all the 6 animals has same sequence pattern and SNP was not revealed in exon-3 region of leptin gene. Al-Sharif *et al* (2022) reported no variation in coding region of leptin gene in dromedary she-camels. Piri *et al* (2018) reported transitional substitutions during comparative nucleotide sequence analysis of Sistan and Baluchistan population of camel. Nobari *et al* (2021) observed length and the difference on sequence of exons of the leptin gene when Turkmen camel gene sequence compared with other types of camels. However, Mahmoud *et al* (2014) observed two non-synonymous SNPs in exon-3 region of leptin gene in Najdi sheep.

This study revealed that studied region lacks SNP and thus monomorphic in nature. Monomorphic pattern showed the conserved nature of leptin gene in dromedary camel. Tahmoorespur and Shojaei (2013) amplified 471bp fragment of leptin gene and also reviewed by Ramadan and Inoue-Murayama (2017). They observed no genetic variation among the 25 Iranian native camels, and no polymorphism in dromedary and bactrian camels. However, Dubey *et al* (2012) reported 3 patterns in both exon-2 and exon-3 of leptin gene using PCR-SSCP followed by nucleotide sequencing in Mithun (*Bos Frontalis*). Mahrous *et al* (2020) revealed monomorphic pattern using PCR-RFLP followed by sequence analysis reported 4 SNPs in Egyptian river buffaloes. It is concluded that polymorphisms reported in other species which have association with economic traits of animals in previous referenced studies whereas no polymorphism is reported in present studied region of leptin gene. Further studies are required with recommendation of large sample size to investigate the relationship between polymorphisms of exon-3 region of leptin gene and the performance traits that may be useful in marker assisted selection (MAS).

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