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GENETIC POLYMORPHISMS OF THE LEPTIN GENE IN EXON 3 OF DESERT SHEEP IN SOUTH DARFUR STATE

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ABSTRACT

Leptin hormone is primarily expressed in adipose tissue and plays a significant role in regulating various functions, including growth, puberty, reproduction, and milk production in both animals and humans. Polymorphisms in the LEP gene have been observed and correlated with a variety of reproductive and productive traits in several sheep breeds worldwide. The objective of this study was to identify polymorphisms in the Leptin (LEP) gene in Sudanese Desert Sheep.

A total of 60 blood samples were collected from the jugular veins of the Sudanese Desert Sheep, and genomic DNA was isolated and purified using a commercial kit (G-spin™ Total DNA Extraction Kit, INTRON). DNA was then amplified using a thermocycler. Polymerase chain reaction (PCR) was carried out to amplify a 471 bp fragment of exon 3 of the leptin gene. Genotyping was performed using the PCR-RFLP technique. PCR products were digested with the OliI restriction enzyme for allelic variant detection in the Sudanese Desert Sheep.

The results revealed polymorphisms in exon 3, resulting in two bands (384 bp and 87 bp) for homozygous GG, three bands (471 bp, 384 bp, and 87 bp) for heterozygous GT, and one band (471 bp) for homozygous TT individuals. These preliminary findings suggest that these polymorphisms may have a significant impact on the improvement of productive traits in the Sudanese Desert Sheep. However, further research with larger sample sizes and DNA sequencing and analysis using advanced molecular techniques is needed, particularly to explore the potential impact of these genetic variants on the Sudanese Desert Sheep meat traits.

Keywords: Genetic variance, leptin gene, desert sheep, South Darfur, Sudan.

1. INTRODUCTION

In a developing country like Sudan, small ruminants, particularly sheep, play an important role in sustainable livestock production and make a valuable contribution. Especially in areas where crop and dairy farming are not economical, sheep significantly support the livelihood of economically weaker sections of society, Jelocnik *et al.*, (2019). However, Sudan desert Sheep, a prominent breed native to Sudan, plays a critical role in the livestock sector, contributing to the livelihoods of local farmers and communities in the arid regions of South Darfur State. As with many livestock species, the genetic characteristics of Desert Sheep are crucial to understanding their adaptability, health, and production potential in harsh environments. Among the various genes influencing these traits, the leptin gene has garnered significant attention due to its role in regulating energy balance, metabolism, and fat deposition in mammals. Leptin, a protein

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hormone predominantly secreted by adipose tissue, has been linked to critical biological processes such as appetite regulation and reproductive health. Leptin is a 16 kDa non-glycosylated protein hormone of the cytokine family, which plays an important role in body growth by maintaining the balance between food intake and energy expenditure through signaling to the brain Friedman and Halaas (1998). The leptin hormone, encoded by the LEP gene, is involved in many biological and physiological processes in the body. The LEP gene, which has three exons and two introns, is located on the 5th chromosome in sheep Javanmard *et al.*, (2008). Understanding the genetic variability of the leptin gene in Desert Sheep can offer valuable insights into breed-specific traits, such as feed efficiency, body condition, and overall productivity, particularly in the challenging environments of South Darfur State. Genetic studies of this nature enhance breeding programs and provide a framework for sustainable livestock management in regions where resources are scarce and environmental stress is high. This study aims to explore the genotypic diversity of the leptin gene in Desert Sheep from South Darfur, contributing to the growing body of knowledge on the genetic factors influencing their performance in such an ecosystem.

2. MATERIALS AND METHODS

Collection of blood samples

Sixty (60) blood samples were randomly collected from Sudanese Desert sheep (40 females, 20 males) from different localities (Bielel, Al Mallam, Labado, OM Gerdood, and Al-Bangadeed), as well as from local livestock markets (Al Mawashi and Al Seref). Five milliliters of blood were drawn from the jugular vein of each sheep and collected in an EDTA Vacutainer tube. The blood samples were then stored on ice, transported to the laboratory, and kept at –20°C.

DNA extraction

Genomic DNA was isolated and purified using a commercial kit (G-spin™ Total DNA Extraction Kit, INTRON). The quality and concentration of the DNA were assessed by electrophoresis on a 2% agarose gel.

PCR amplification

A 471 bp fragment containing exon 3 of the leptin gene was amplified by PCR using the following primers: forward 5′-AGGAAGCACCTCTACGCTC and reverse 3′-CTTCAAGGCTTCAGCACC. The PCR reactions were performed in a final volume of 20 µL using a Maxima™ PCR cocktail containing: i-Taq PreMix Kit (2.5 U Taq polymerase, 2.5 mM dNTPs, 1x reaction buffer, 1x gel loading buffer). Then, 2 µL of genomic DNA, 1 µL of each primer (forward and reverse), and 16 µL of sterilized distilled water were added. The amplification cycles were carried out in a PTC-100 thermocycler. Reaction conditions were as follows: initial denaturation at 94°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 1 minute, annealing at 58°C for 1 minute, and extension at 72°C for 1 minute. A final extension step was performed at 72°C for 5 minutes.

Restriction fragment length polymorphism (RFLP)

PCR products were digested with the restriction enzyme OliI at 37°C for 4 hours, using 1 U of OliI, 10 µL of PCR product, 2 µL of 10x Buffer R, and 17 µL of nuclease-free water. The

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digestion products were resolved on a 3% agarose gel and a 100 bp DNA marker for 70 minutes at 80 V.

3. RESULTS AND DISCUSSION

The quality of extracted DNA was assessed by 0.5% agarose gel electrophoresis. The 471bp fragment comprising the exon 3 region of the LEP gene was successfully amplified. The locus-specific clear dark bands were visualized in all samples (Fig.1). For detection of allelic variation in the amplified exonic regions of LEP, the PCR products were further processed for RFLP. The PCR product was digested with *olil* restriction enzyme for detection of SNP. The digested products were resolved in 3% agarose gel, resulting in two bands (384 bp and 87 bp) as homozygous GG, three bands (471 bp, 384 bp, and 87 bp) as heterozygous GT, and one band (471bp) as homozygous TT individuals (Fig 2).

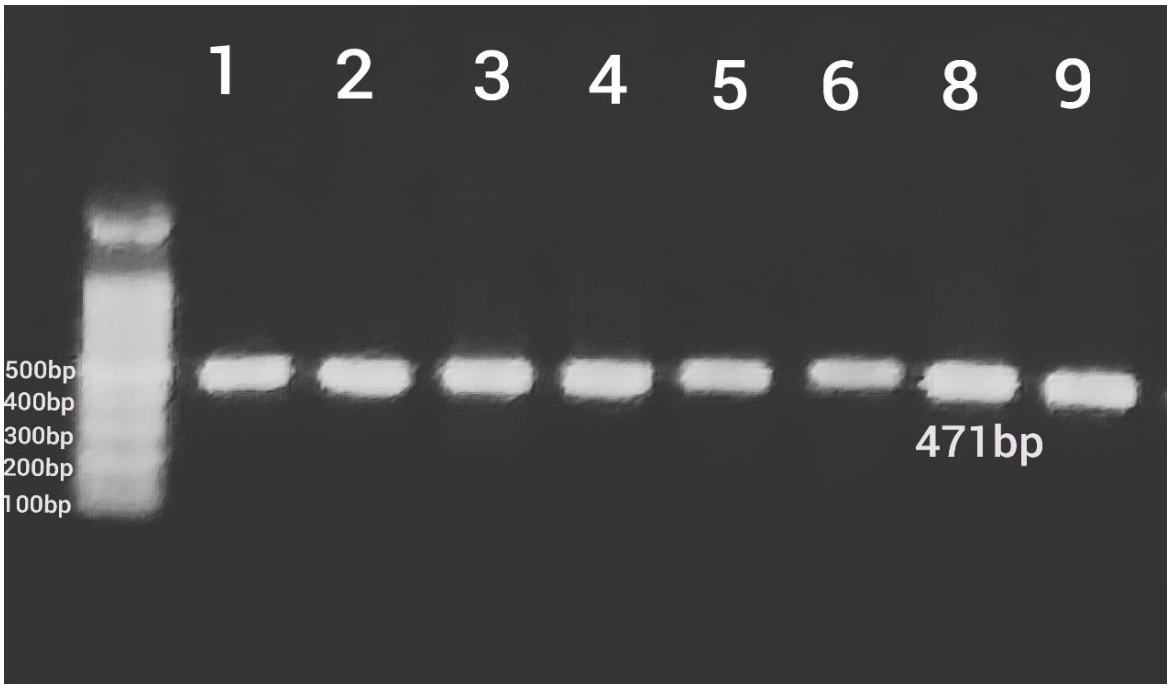

Fig 1: PCR amplicons of Leptin gene in Desert sheep

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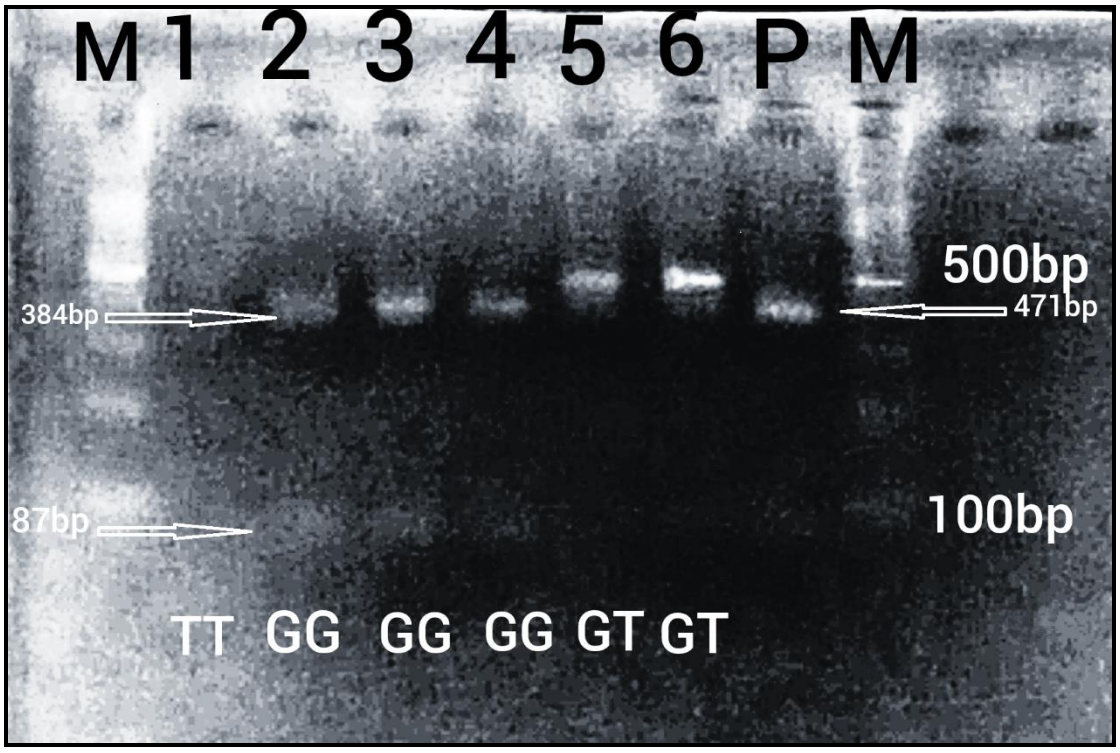

Fig 2: PCR-RFLP profile of the leptin gene exon 3 digested with the OliI enzyme on a 3% agarose gel. Lane 1 represents the TT genotype, lanes 2, 3, and 4 represent the GG genotype, and lanes 5 and 6 represent the GT genotype. P indicates the undigested PCR product, and M is the 100 bp DNA ladder.

The current study aimed to detect possible polymorphisms in the leptin gene within exon 3 in the Sudan Desert sheep breed. The PCR-RFLP technique was used to identify leptin genetic variants. A 471 bp fragment of the leptin gene was screened for this breed. The PCR product was digested with the OliI restriction enzyme to detect potential SNPs. The digested products were resolved on a 3% agarose gel. Digestion of the 471 bp PCR product with OliI restriction endonuclease at the T387G locus resulted in two bands (384 bp and 87 bp) for homozygous GG individuals, three bands (471 bp, 384 bp, and 87 bp) for heterozygous GT individuals, and one band (471 bp) for homozygous TT individuals. Genotyping of the samples was performed based on the presence of the 384 bp and 471 bp bands, which are visible in the gel images. The present findings are consistent with the study by Meena *et al.* (2017), who found that the Malpura sheep breed had one polymorphic SNP (G387T), while the other two SNPs (G271A and C316A) were monomorphic in the studied population. Several studies have reported polymorphism in exon 3 of the leptin gene in Iranian Baluchi and Kermani sheep using the same primers, which detected the three genotypes via the PCR-SSCP technique (Tahmoorespur et al., 2010; Shojaei *et al.*, 2010; Tahmoorespur and Ahmadi, 2012).

Additionally, Zhou *et al.* (2009) reported that the exon 3 region of the leptin gene was polymorphic in six New Zealand sheep breeds (Romney, Merino, Coopworth, Corriedale, Poll

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Dorset, and Suffolk). They identified four SNPs across all breeds, of which the 271 bp, 316 bp, and 387 bp SNPs were nonsynonymous, resulting in amino acid changes. However, Makoei sheep of Iran exhibited five genotypes in exon 3 of the leptin gene, similar to the findings in New Zealand sheep breeds Hashemi *et al.* (2011) and Sadeghi *et al*. (2014). The exon 3 of the leptin gene in Assaf, Awassi, and Dorper sheep breeds had synonymous and non-synonymous mutations Reicher *et al*. (2010). Moreover, Cauveri *et al*. (2014) identified two novel SNPs in the untranslated regions (UTRs) of exon 3 of the leptin gene in Nilagiri sheep. Nilagiri sheep had only one SNP (SNP-L1) that was monomorphic (AA genotype), while the second SNP (SNP-L2) was polymorphic. The Nilagiri sheep breed did not report the four SNPs described by Zhou *et al*. (2009). The five single-strand conformation polymorphism (SSCP) genotypes of the Makooei sheep breed were found to be significantly associated with some body measurements such as heart girth and rump length, while body length, height at the back, and scrotal circumference were not affected by the genotypes Sadeghi *et al*. (2014).

4. CONCLUSION AND RECOMMENDATIONS

The preliminary findings indicate that these polymorphisms could play a significant role in improving productive traits in the Sudanese Desert Sheep. However, to better understand the potential effects of these genetic variants, particularly on meat traits, further research is necessary. This should involve larger sample sizes and advanced molecular techniques, such as whole genome sequencing and analysis.

Ethical approval

Due to the ongoing war in Sudan, we were unable to obtain ethical approval from the Research Ethical Approval Committee at the University of Nyala. However, the guidelines and regulations established by the Sudan Veterinary Council were strictly followed during animal handling and sampling.

Conflicts of Interest:

The authors declare no conflicts of interest for this piece of scientific contribution.

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Authors contributions:

Khalid Tamim collected the samples, conducted the experiments, and wrote the first draft of the manuscript. Alfatih and Abass revised the manuscript, while Hamza supervised the scientific article.

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