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Short communication

PCR-RFLP ANALYSIS OF INSULIN-LIKE GROWTH FACTOR-1 GENE OF KALAHARI RED GOATS

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ABSTRACT

Insulin-like growth factor 1 gene (*IGF-1*) is associated with growth traits in different livestock, but its association with growth traits in South African indigenous goat breeds is poorly understood. The study's objective was to identify the single nucleotide polymorphisms (SNPs) of *IGF-1* in the Kalahari Red goat breed and their association with the growth traits. Two genotypes (KK and KM) were identified using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The frequency of genotype KK and KM was 0.56 and 0.44 respectively. The allelic frequency of allele K and M was 0.78 and 0.22 respectively. The studied Kalahari Red goat breed population was not in Hardy Weinberg equilibrium (HWE) as revealed by the Chi-square test ($\chi 2 = 0.39$). Marker-trait association indicated that the identified genotypes had no association ($P > 0.05$) with the measured growth traits. In conclusion, the current study suggests that two identified genotypes of *IGF-1* might not be used as potential genetic markers during selection to improve growth traits. Further studies need to be conducted on SNPs of *IGF-1* and their association with growth traits using a larger sample, more growth traits and targeting more exons of Kalahari Red goats.

Keywords: Single nucleotide polymorphisms, Body weight, Genetic markers, Hardy Weinberg equilibrium

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INTRODUCTION

Kalahari Red goat is a meat type goat with a red medium to the large framed body, round horns which bend backwards and loose skin with folds (Snyman, 2014). This breed is hardy, naturally adapting and less susceptible to diseases and parasite infestation as compare to other goat breeds (Sanni *et al.,* 2018). It is also known for its rapid growth, giving birth to twins or triplets at a time and producing enough milk to support its kids (Amie Marini *et al.,* 2012). However, this breed still requires the genetic improvement of growth traits to increase meat production (Ssewannyana *et al.,* 2004; Bhattarai *et al.,* 2019). Othman *et al.* (2016) indicated that productivity improvement can be obtained with the use of new genetic technology like selecting traits through marker-assisted selection. Insulin-like growth factor -1 gene (*IGF-1*) is among the genes identified for improvement of productivity (Al-Qasimi, *et al.,* 2019). *IGF-1* is a component of the somatotrophic axis which plays a key role in the growth and metabolism of animals (Lestari *et al.,* 2020). The *IGF-1* was mapped on chromosome 5 with a sequence of 6.784 bp (D26119), made up of leader exons (1w, 1, and 2) and exons (3, 4, and 6) (Zhang *et al.,* 2008; Lestari *et al.,* 2020).

It was discovered that there is an association of genetic polymorphisms of the *IGF-1* in Korean native Ogol chickens with growth traits (Seo *et al.,* 2001), in M1 and M3 lines of Beef-booster Inc. (Calgary, Canada) (Li *et al.,* 2004), and in Malabari and Attappady Black goat breed (Naicy *et al.,* 2017). Kotze et al. (2004) used microsatellite markers to investigate the genetic profile of the Southern African Kalahari Red goats and provided the genetic characterization for the future management of the breed. However, potential genetic markers of the *IGF-1* gene and its association with growth traits in South African Kalahari Red goats are not known. Hence, the objective of the study was to identify the single nucleotide polymorphisms (SNPs) of *IGF-1* Kalahari Red goats and their association with the growth traits. The study will help Kalahari Red goat farmers during breeding for selection based on genetic markers to improve growth traits.

MATERIALS AND METHODS

Study area: The Zuurfontein farm which is situated under Polokwane Local Municipality, Limpopo province, South Africa was used in conducting the study. The farm lies at latitude -23.5766^0 S and longitude 29.5209^0 E, 1154m above sea level. The ambient temperature ranges between 16^{0}C to 28.1^{0}C in summer (December to February), 7^{0}C to 21^{0}C in winter (June to August) and an annual rainfall of more than 600mm (Shabalala *et al.,* 2019). The laboratory work was conducted at the Department of Biochemistry, Microbiology and Biotechnology, University of Limpopo, Limpopo Province, South Africa.

Ethical approval: All procedures were performed following the standards and protocols set by the Animal Research Ethics Committee (AREC) project number AREC/14/2021: PG at the University of Limpopo.

Experimental animal and management: A total of fifty (n = 50) Kalahari Red goats were used in the study, all the animals were between two to three years of age. The Kalahari Red goats were reared under an extensive rearing method. The goats were kept in the kraals during the night and released into the veld to feed on the different tree leaves available on the farm during the day. However, the males were kept in pens and were fed a standard ration bought from a feed company (NTK, Polokwane, Limpopo province of South Africa). The goats were dosed on a monthly based and dipping took place every Wednesday each week. The males and females were kept in separate pens. The kraals had a water pen and clean water was provided *ad-libitum*. Ear tags were used as a method of identification.

Blood sampling and DNA extraction: A total of fifty (n = 50) blood samples were collected from Kalahari Red goats at the Zuurfontein farm. A 3mL Luer slip-tip syringe was used to collect blood from the jugular vein and the blood was put into anticoagulant (EDTA) blood tubes awaiting DNA analysis. The procedure of blood collection was performed by the university veterinarian and samples were kept at 4 degrees Celsius till use. The DNA was extracted and purified as indicated by Noegen's Genomic DNA isolation kit protocol (Neogen, Lansing, Michigan, USA).

DNA Amplification: *IGF-1* was amplified using Polymerase chain reaction (PCR). Primers to amplify the *IGF-1* were designed based to the sequence in the National Centre for Biotechnology Information (NCBI) database sequences (GenBank accession No. D26118.1) using Primer Premier 5.0 software (PREMIER Biosoft, Palo Alto, CA, USA). Table 1 shows the primers that were used to amplify the *IGF-1*.

Table 1: Primer sequence, amplified region and fragment size for PCR amplification of *IGF-1*.

Amplified Region	Primer sequence (5'-3')	Genbank Accession number	Fragment size Product length (location)	Annealing Temperature
Exon 4	Gctgggtgtagcagtgaaca Gttgcttcagccgcataact	D26118.1	320bp (308 – 627nt)	60°C

PCR mixture of 50 µL, containing 25µL of Master Mix, 1µL of each primer forward and reverse, 5µL DNA template and 18µL deionised double–distilled water. The PCR program was left at 95°C for 5 min to denature, followed by 34 cycles of 94°C for the 30s, 60°C for the 30s, 72°C for 30 s and a final extension at 72°C for 10 min. The PCR product results were separated by electrophoresis on a 1.2% agarose gel. The gel was stained with ethidium bromide visualized and photographed under a U.V. trans-illuminator (Spectroline, New York, USA).

Genotyping: Restriction Fragment Length Polymorphism (RFLP) was employed to genotype PCR products using the *HaeII* enzyme. A total of 50µL reaction mixture consisting of 30µL of the PCR product, 5µL of 10 X buffer, 13µL water and 2 µL of fast restriction enzyme were used to carry out RFLP. The reaction mixture was incubated at 39°C for 24 hours. The restriction digest reaction products were electrophoresed on a 1.2% agarose gel, visualised and photographed using a U.V. trans-illuminator (Spectroline, New York, USA).

Statistical analysis: The POPGENE software (version 1.32, University of Alberta, Canada) for population genetic analysis was used to calculate allele and genotype frequencies as explained by Rashijane et al. (2022). The allele frequencies for Hardy-Weinberg equilibrium were assessed using Chi-square ($\chi 2$). The marker-trait association analysis was determined using the general linear model (GLM) of Statistical Package for Social Sciences (IBM SPSS, 2020) version 26.0 software. The following model was used:

$$Y_{ij} = \mu + G_i + e_{ij}$$

Where:

Y_{ij} = Phenotypic values of traits (live body weight and linear body measurements (Rump height, Body length, Sternum height, Heart girth, Withers height),

μ = Population mean,

G_i = Fixed effect of genotype, and e_{ij} = Random residual error.

RESULTS

Nucleotide sequence amplified analysis

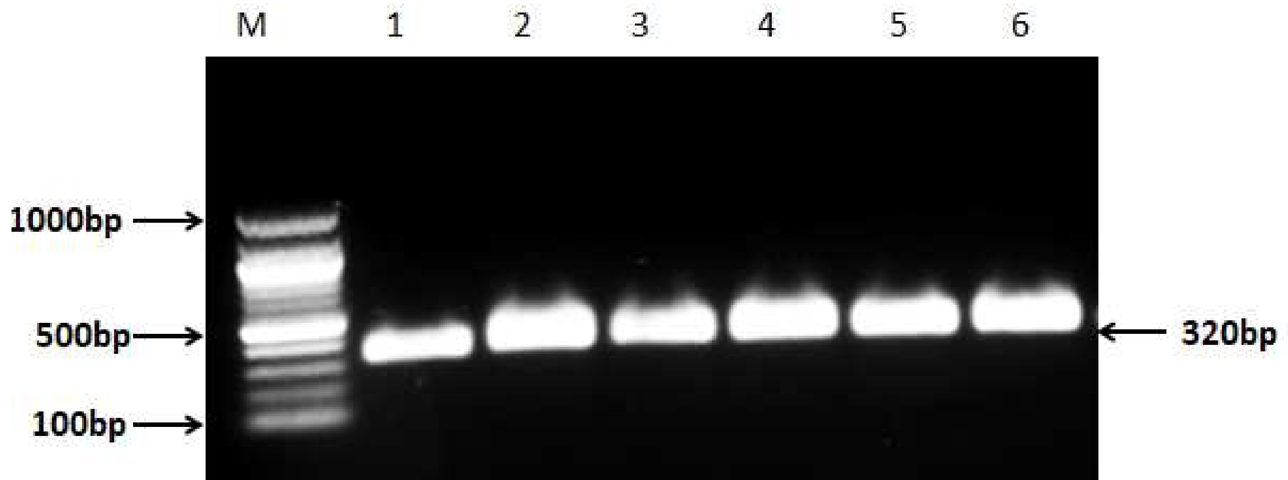

Figure 1: PCR products of *IGF-1* on 1.2% agarose gel. M, DL 1000 DNA marker, 1 to 6 *IGF-1* fragments amplicons

Amplification of DNA was performed using Polymerase Chain Reaction (PCR). The DNA was extracted successfully and the primers were used to amplify *IGF-1* which resulted in the generation of 320bp DNA fragments consisting of the expected size as determined from gene sequence information (Figure 1).

PCR-RFLP analysis

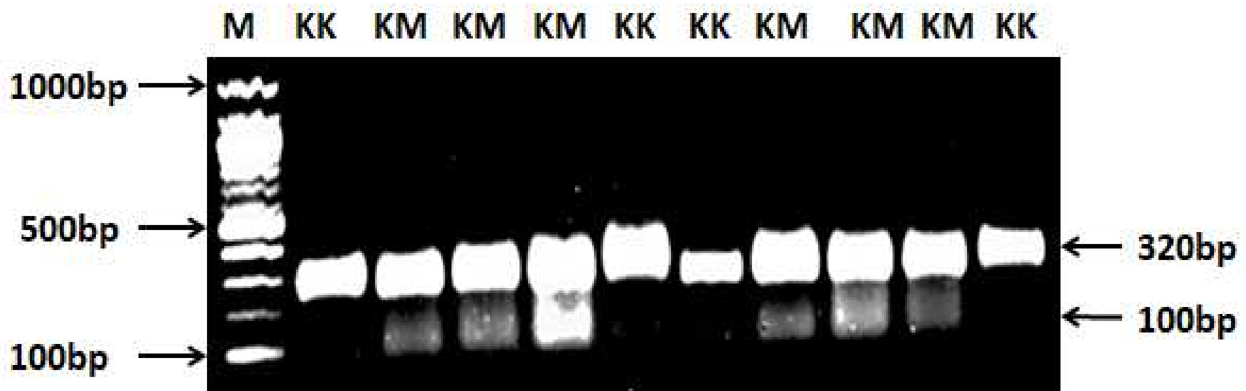

Figure 2: PCR-RFLP for genotyping of *IGF-1*

PCR-RFLP was employed to determine SNP. The resulting gel patterns from PCR-RFLP are presented in Figure 2. PCR-RFLP of amplicons revealed 2 banding patterns. The first pattern showed one band of 320bp and the second pattern showed two bands (100bp and 320bp). There were two genotypes KK and KM identified, KK with one band and KM with two bands.

Allelic and genotypic frequencies: The genotypic and allelic frequencies were determined by POPGENE software. The estimated genotypic and allelic frequencies of *IGF-1* in Kalahari Red goats were determined in the current study (Table 2). Allele K and M were found and K had a high frequency than the M allele. There were two genotypes KK and KM and the frequency of the KK genotype was higher as compared to KM genotype. The genotype distribution differed statistically ($P < 0.05$) from Hardy Weinberg equilibrium (HWE).

Table 2: Allelic and genotypic frequencies at the single nucleotide polymorphism locus of *IGF-1* in Kalahari Red goats.

Genotype	Number of goats	Genotypic frequency	Allele	Allele frequency	X^2
KK	28	0.56	K	0.78	3.98*
KM	22	0.44	M	0.22	

X^2: chi-square test, degree of freedom = 1, P < 0.05.

Polymorphism parameters: POPGENE software was used to determine the polymorphism parameters as presented in Table 3. The results indicated that there was high homozygosity of *IGF-1* in Kalahari Red goats and low heterozygosity. Based on the results of PIC, it was revealed that Kalahari Red goats possessed medium polymorphism (0.25 < PIC < 0.5) at *IGF-1*.

Association of genetic variants with growth traits: The association between genotypes and growth traits was determined using GLM. To investigate the effect of *IGF-1*, the association between genotype and withers height, rump height, body length, heart girth, sternum height and body weight was analysed. The outcomes of the association are shown in Table 4. There was no statistical association between the genotypes and the growth traits measured in Kalahari Red goats.

Table 3: Polymorphism parameters.

Gene homozygosity (H_0)	Gene heterozygosity (H_e)	Effective Allele number (N_e)	Polymorphism information (PIC)
0.66	0.34	1.52	0.28

Table 4: Association between the polymorphism in *IGF-1* and body measurement traits of Boer goats.

Traits	*IGF-1* Genotypes KK (n = 28) (Mean ± SE)	KM (n = 22) (Mean ± SE)	F value	Significance
Body weight (kg)	47.82 ± 1.835	53.45 ± 1.79	0.61	0.44
Rump height (cm)	66.59 ± 1.20	72.68 ± 0.92	0.57	0.45
Body length (cm)	76.41 ± 1.47	84.12 ± 1.41	0.91	0.35
Sternum height (cm)	38.82 ± 1.01	44.36 ± 0.51	0.01	0.91
Heart girth (cm)	81.91 ± 1.67	90.96 ± 1.63	0.64	0.43
Withers height (cm)	66.64 ± 1.05	65.93 ± 2.43	1.66	0.20

SE: standard error

DISCUSSION

The finding of the study recorded SNPs on exon 4 of *IGF-1* in Kalahari Red goats. The outcomes of the study are in line with the study that was conducted by Sarmah *et al.* (2019) which reported a SNP at 5752bp position with nucleotide transversion from G to C in Assam hill goats. The research conducted by Lestari *et al.* (2020) in Kejobong goats reported a SNP observed at intron 4 as a transversion, these results are contrary to the current results. The evaluation of *IGF-1* gene polymorphism in Egyptian small ruminant breeds (sheep breeds; Barki, Ossimi and Rahmani, goat breeds; Baladi, Barki and Zaraibi) revealed a SNP (C > G) at position 282 (Othman *et al.,* 2016). The sequencing analysis of Mongolia cashmere goats by Liu *et al.* (2012) revealed a transition from C > G at nucleotide 69, which caused a missense mutation in exon 2. The results of the current study suggest that there was a variation of the *IGF-1* in Kalahari Red goats.

The results obtained from chi-square indicated that the population used in the current study is not under Hardy-Weinberg equilibrium (HWE). The current results were in line with the study of Lestari *et al.* (2020) which indicated that the Kejobong goat genotype was different from HWE. Furthermore, the findings are in harmony with the result of Rasouli *et al.* (2017) in Markhoz goats revealed that the allelic and genotypic distributions for *IGF-1* and insulin-like growth factor binding protein 3 genes (*IGFBP-3*) were found statistically different from Hardy Weinberg equilibrium. The current study suggests that the allelic and genotypic frequencies of *IGF-1* changed over time in the Kalahari Red goats. This may be due to mutations and genetic drift. Zhang *et al.* (2008)

reported that the chi-square test on two genotypes within the Nanjiang Huang goat population was not in Hardy-Weinberg equilibrium.

The results on the association of genetic variants with growth traits showed that the genotypes KK and KM had no significant relationship with all the growth traits in Kalahari Red goats. The findings are in agreement with the findings of Sarmah *et al.* (2019) which highlighted that even though there was SNP on *IGF-1* in Assan hill goats, there was no association between the genotypes and the body weight. Contrary to the findings of the current study, Zhang *et al.* (2008) reported that *IGF-1* polymorphism had an association with body weight in Nanjiang Huang goats, with goats which had genotype CC being heavier than those with genotype GC and GG. The study of Lestari *et al.* (2020) found that animals with genotype GG were significantly larger and heavier than those with genotype CC. Othman *et al.* (2016) reported an association of *IGF-1* polymorphism with different growth traits in Egyptian small ruminant breeds (sheep breeds; Barki, Ossimi and Rahmani, goat breeds; Baladi, Barki and Zaraibi). The results of the study suggest that both genotypes KK and KM cannot be used as potential genetic markers in Kalahari Red goats for improvement of growth traits, because measured traits might be influenced by other genes and the environment. Sarmah *et al.* (2019) highlighted that lack or no association might be due to the occurrence of mutations which affected the regulation of protein.

In the past, breeding for growth traits relied much on the phenotypic measurements and performance records, this led to less improvement which took a long time to achieve due to some key traits having low heritability (Ekegbu *et al.,* 2019). Hence, the studies conducted by EL-Magd *et al.* (2017) in buffalo, Lazar *et al.* (2018) in the Carpatina breed, and Abdalhag *et al.* (2015) in Jinghai yellow chickens were aimed at improving animal breeding using phenotype and genetic markers. However, more research needs to be conducted on the association of single nucleotide polymorphisms of *IGF-1* and growth traits in Kalahari Red goats using a larger sample size and including the DNA sequencing to confirm the single nucleotide polymorphisms identified, the growth traits included should be increased.

Conclusion: *IGF-1* exon 4 amplified with specific primers was found to be polymorphic on restriction digestion with *HaeII* enzyme using PCR-RFLP and revealed two genotypes KK and KM with frequencies of 0.56 and 0.44. The observed genotypes were not associated with the studied traits. Further studies are required to use a larger sample size.

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Authors' contribution statement: KM, TLT and VM designed the experiment, KM analysed the data. TLT, KM and VM performed fieldwork and compiled the manuscript. TLT revised and edited the compiled manuscript. All authors approved the final manuscript.

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