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Genetic Polymorphism of Growth Hormone Gene in Dorper Sheep using PCR-RFLP

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ABSTRACT

Background: The growth hormone gene has a significant impact on postnatal longitudinal growth and development as well as the metabolism of protein, carbohydrates, and fats moreover its effects indirectly relate to bone and skeletal development. The objective of the study was to identify a single nucleotide polymorphism of the growth hormone gene and its association with growth traits in Dorper sheep.

Methods: Data were collected from 50 Dorper sheep of 1 to 2 years of age at the University of Limpopo Experimental farm. The objective of the study was achieved by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism and General Linear Model (GLM).

Result: PCR-RFLP reports revealed different segment patterns resulting in two genotypes such as AA and AB genotypes. Marker-traits association analysis showed that no significant difference was observed between AA and AB genotypes regarding all the measured traits except with wither height and AA genotype was remarkably related to higher withers height than AB genotype. It can be concluded that *GH1* could be used as a genetic marker for marker-assisted selection (MAS) during breeding for the improvement of withers height.

Key words: Exon 4, Genetic selection, Marker-trait association, Restriction fragment length polymorphism.

INTRODUCTION

Dorper sheep is a South African breed that resulted from the crossbreeding of Dorset Horn and Blackhead Persian sheep (Joy *et al*., 2020). According to Issakowicz *et al*. (2018), this breed is distinguished by excellent productivity, good body shape, and carcass related to meat quality. They have been identified as the second most popular sire breed for meat characteristics in South Africa by national and international producers because they produce meat with greater tenderness and milder flavor than wool sheep breeds Villatoro, (2021) and are typically raised under extensive management (Cloete *et al*., 2013).

Villatoro *et al*. (2021) classified Dorper sheep as an adaptable fast-growing animal that produces heavy meat resulting in appealing cuts for retailers and consumers, and Shackelford *et al*. (2012) stated that they can withstand dehydration and drought. However, sheep farmers have a strong desire for heavier sheep, which is why the growth performance of sheep needs to be improved to meet customer demand and requirements (Rajni *et al*., 2014).

According to Zhao *et al*. (2015), traditional genetic selection methods improve economic important traits such as body weight significantly however, traditional methods are time consuming. Therefore, the candidate gene approach was introduced as a powerful tool in which the researchers recognize, map and analyse single nucleotide polymorphism (SNPs) of genes such as growth hormone gene (*GH1*) associated with the traits of interest (Valencia *et al*., 2022). Al-Sharif *et al*. (2022), indicated that *GH1* has been the candidate gene employed in much research since it is connected with food partitioning, which contributes to rapid growth and high performance of the animals.

According to Rajni *et al*. (2014), the utilization of Marker-Assisted Selection (MAS) helps livestock breeders in achieving more precise selection aims. The growth hormone gene has been utilized as a marker in numerous species, including goat (Mohammadabadi, 2012), cattle (Kumari *et al*., 2014), chicken (Nguyen *et al*., 2019) and sheep (Bayraktar and Shoshin, 2022) and its polymorphism has been linked with carcass weight as well as production qualities (Depison *et al*., 2017). However, the *GH1* polymorphism and association with growth traits in Dorper

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sheep is not yet known. Hence, the objective of the study was to identify the single nucleotide polymorphisms of *GH1* and their relationship with growth traits in Dorper sheep.

MATERIALS AND METHODS

The current study was carried out at the University of Limpopo's experimental farm, which is located 10 kilometers west of the university, to measure growth traits. The farm has semi-arid climatic conditions, with winter temperatures ranging from 5°C to 28°C and summer temperatures ranging from 10°C to 36°C and an annual rainfall of less than 400 mm (Kutu and Asiwe, 2010). The laboratory work was carried out in the Department of Biochemistry, Microbiology, and Biotechnology at the University of Limpopo in Limpopo Province, South Africa.

The study was conducted using 50 Dorper sheep between the age of 1 and 2 years. The animals were subjected to be raised extensive farming system. *Ad libitum* access to clean water was provided. To prevent all possible diseases, vaccination and dipping programs were carried out regularly before lambing, weaning, and breeding. Body length (BL), heart girth (HG), sternum height (SH), withers height (WH), rump height (RH) and BW (body weight) of Dorper sheep were take following the recommendations of Birteeb *et al*. (2012) to correlate the growth hormone gene. Blood samples (2-3 ml) were collected from the external jugular veins of each Dorper sheep (n=50) at a once-off interval by a veterinarian using 21-gauge needles and 5 ml syringes. Blood samples were collected into 10 mL EDTA-coated tubes and stored at 4°C until use. Following the manufacturer's protocol, DNA was isolated and purified from blood samples using Norgen's Genomic DNA Isolation kit (Norgen Bioteck Corp, Canada). A UV spectrophotometer (Thermo Fisher Scientific, India) was used to determine the purity and concentration of DNA samples, while agarose gel electrophoresis was used to determine their integrity.

The growth hormone gene was amplified using PCR. Primers to amplify the growth hormone gene were designed using Primer Premier 5.0 software based on the sequence in the National Centre for Biotechnology Information (NCBI) database sequences (GenBank accession No.GQ452268) (PREMIER Biosoft, Palo Alto, CA, USA). Table 1 lists the primers used to amplify the growth hormone gene.

PCR reaction was carried out in a 50 µl reaction mixture containing 7 µl of genomic DNA, 25 µl of master mix, 1 µl of each forward and reverse primer and 16 µl of de-ionised double-distilled water. PCR amplification was carried out following the thermal cycling conditions as described by Gorlov *et al*. (2017): Pre-denaturation at 95°C for 5 min and then 33 cycles of 95°C for 45s for denaturing, 60°C for 45s for annealing, 72°C for 45s for extension and final synthesis at 72°C for 10 min. The resulting products were separated by electrophoresis on 1.2% agarose gel stained with ethidium bromide, visualised and captured under a UV trans-illuminator (Spectroline). Restriction fragment length polymorphism (RFLP) was used for genotyping PCR products. PCR products were digested with *HaeIII* restriction endonuclease and electrophoretically separated. For RFLP analysis, 20 µl of PCR products, 5 µl of PCR buffer, 23 µl of distilled water and 2 µl of *HaeIII* enzyme together to make a total of 50 µl were incubated in the Thermo scientific for 20 hours at 36.9°C. The restriction digestion solutions were mixed with 4 µl of loading dye, then loaded on 1.2% agarose gel stained with ethidium bromide, visualised and photographed by U.V. trans-illuminator (spectroline). PCR was used for DNA amplification and PCR-FRLP was used to determine single nucleotide polymorphisms. General Linear Model (GLM) was performed for Marker-Trait Association analysis. The following GLM model of SAS 9.4 software (SAS, 2019) was used for marker-trait association analysis:

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

Where:

Y_{ij} = Phenotypic values of ith trait on j^{th} genotype.

μ = Population mean.

G_i = Fixed effect of ith genotype.

e_{ij} = Random residual error.

Genetic equilibrium of the population under Hardy- Weinberg theorem was measured using Chi-square test and for Population Genetic Analysis, POPGENE version 1.32 software was used to calculate Allelic and genotypic frequencies.

RESULTS AND DISCUSSION

DNA amplification and restriction fragment length polymorphism (RFLP) analysis of the growth hormone gene

A segment of exon 4 of the *GH1* of Dorper sheep was amplified using PCR to determine the size of the gene. A 934 bp PCR amplicon size was obtained as shown in Fig 1(A). A total of 50 Dorper sheep *GH1* amplified products were genotyped with PCR-RFLP analysis to detect the polymorphism on exon 4. The PCR-RFLP electrophoretic results as presented in Fig 1(B) revealed different band patterns, some with one band and the others with two bands. In all samples of Dorper sheep assessed in this study, PCR-RFLP recognised two genotypes (AA and AB) in the coding region of the *GH1*.

Reports by Abdelmoneim *et al*. (2017) are in accordance with the current study on 100 Harri sheep where a novel single nucleotide polymorphism in exon 4 was discovered. However, the same author discovered another SNP (G871A) on intron 2 and another SNP on intron 4 at position 1383.

Table 1: Primer information used for growth hormone *gene* amplification.

Primer	Sequence	Annealing temperature (°C)
Forward	GGAGGCAGGAAGGGATGAA	60
Reverse	CCAAGGGAGGGAGAGACAGA	

Contrary to our results was reported by Cauveri *et al*. (2016) on study conducted on the single nucleotide polymorphisms in *GH1* associated with growth traits in Nilagiri sheep of Tamil Nadu, they analysed the complete gene and found no SNP in all exons, disagreement could be due to differential expression of genes which influences animal's physiology, however, two SNP(s) were noted on transition G/A on position 480 and transition G/A on position 871 in intron 1 and intron 2 respectively.

Genotypic and allelic frequencies

Population genetic analysis was used to calculate allelic and genotypic frequencies of the studies population and genetic equilibrium of the population under Hardy-Weinberg theorem was measured using chi-square test. Allelic and genotypic frequencies for the *GH1* single nucleotide polymorphism are presented in Table 2. Two alleles (A and B) and two genotypes (AA and AB) were noted where frequency of allele A was found to be higher than of allele B, moreover genotypic frequency of genotype AA was also found to be higher than of genotype AB. Chi-square test showed that the population's genotypic and gene frequencies were similar to the expectations of Hardy-Weinberg (HWE) (X^2=1.56). Chi-square test results demonstrated that the allelic and genotypic frequencies *GH1* SNP are under Hardy-Weinberg equilibrium, suggesting that the population's gene and genotypic frequencies remain constant from generation to generation.

Our chi-square results are in accordance with chi-square reports on study conducted by Han *et al*. (2016) which demonstrated the population to be under HWE in OT sheep while other individual gene and genotypic frequency deviated from HWE. The current study suggest that the studied population is under HWE implying that the genotypic and allelic frequency of *GH1* of Dorper population does not change from generation to generation additionally the change in the DNA sequence does not change the protein structure and function.

Polymorphism information analysis

Polymorphism information analysis and genetic diversity of the population were achieved by Population Genetic Analysis. Growth hormone gene genetic diversity parameters (H_o, H_e, N_e and PIC) for the SNP are shown in Table 3. Outcomes revealed that gene homozygosity was lower than gene heterozygosity with the effective allele number of 1.34 and lower polymorphism information content.

SNP genotypes of growth hormone gene associated with growth traits

A general linear model (GLM) was performed for marker-trait association. Association between marker-traits are displayed in Table 4. Reports showed that no significant difference (P>0.05) was observed between AA and AB genotypes regarding BW, RH, BL, SH and HG. T735A (*GH1*)

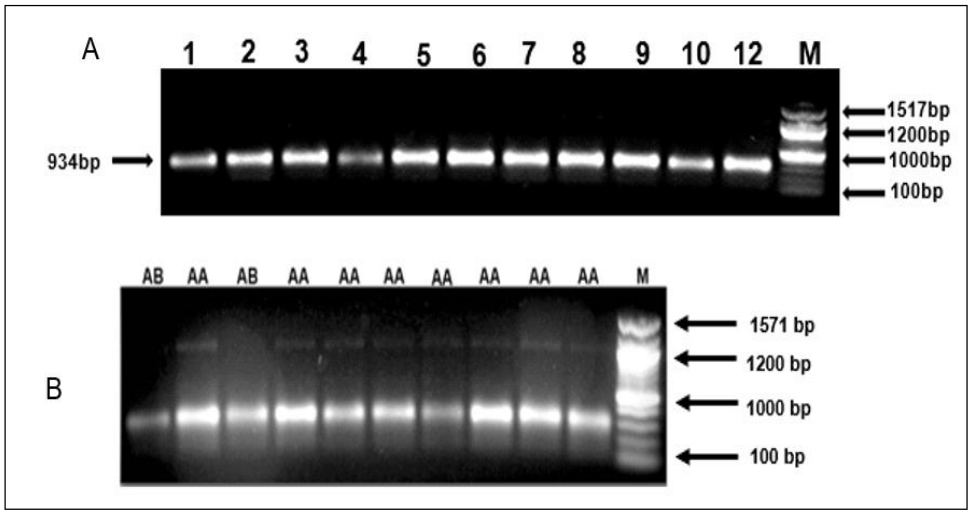

Fig 1: PCR products from the amplification of exon 4 region of *GH1* gene in Dorper sheep (A). PCR-RFLP band patterns of exon 4 PCR product of the growth hormone gene in Dorper sheep (B).

Table 2: Genotypic and allelic frequencies at the single nucleotide polymorphism locus of *GH1* in Dorper sheep.

Genotype	Number of animals	Genotypic frequency	Allele	Allele frequency	χ^2
AA	35	0.7	A	0.85	1.56^{ns}
AB	15	0.3	B	0.15	

*P<0.05: Statistically significant when the data were analysed using a Pearson's goodness-of-fit chi-square test (degree of freedom= 1), ^{ns}: non-significant (P<0.05).

Table 3: Polymorphism information analysis of growth hormone gene of Dorper sheep.

Gene homozygosity p (H_0)	Gene heterozygosity (H_e)	Effective allele number (N_e)	Polymorphism information content (PIC)
0.74	1.56	1.34	0.22

Table 4: Association of the polymorphism of *GH1* with growth traits of Dorper sheep.

Traits	AA (n=35) (Mean±SE)	AB (n=15) (Mean±SE)
Body weight (kg)	33.17±1.19	33.47±1.41
Rump height (cm)	62.46±0.83	62.27±0.88
Body length (cm)	68.97±1.04	67.33±1.66
Sternum height (cm)	40.97±0.51	41.87±0.73
Withers height (cm)	61.06±0.61[a]	57.53±1.55[b]
Heart girth (cm)	83.83±1.63	84.00±1.47

SE: Standard error, n: Number of animals. Different superscript on the same row shows significant difference (P<0.05).

showed to have a statistical relationship with WH (P<0.05), genotype AA was remarkably related to higher WH than genotype AB.

Gorlov *et al*. (2017) results on the association of the growth hormone gene polymorphism with growth traits in Salsk sheep breed are not in parallel with the current study, they discovered 3 genotypes (AA, AB and BB) and AB genotype was associated with positive effect on carcass weight. Furthermore, Malewa *et al*. (2014) on sheep of Donggala and East Java breeds and Hajihosseinlo *et al*. (2013) on Makooei sheep breed revealed the presence of genotype AB result with high impact on carcass weight.

Disagreement towards the current study was reported by Malewa *et al*. (2014) on Indonesia fat tailed sheep, they reported that *GH1* polymorphism affect growth traits such as weaning weight in both sheep breeds as it was noted that in Donggala sheep, genotype AA had a significant on growth traits than genotype BB, moreover same study noticed higher significance of genotype AB than genotype AA and BB on growth traits of East Java sheep. Disagreements might be due to use of different sheep breeds.

Report by Moradian *et al*. (2013) in Makooei sheep is in accordance with current study that there was no association between SNP genotypes and body weight. Concerning the results found on our association, genotype AA in the *GH1* might be used as the potential genetic marker when improving withers height. Presence of association implies that the genotype of polymorphism has a chance of occurring more often than expected by chance in an organism having position of the trait (Abousoliman *et al*., 2021).

Gene polymorphisms serve as the potential approach in improving major economically essential traits such as growth traits as they are regarded as aspects to evaluate animal economic value (Han *et al*., 2016) hence more breeders improve molecular genetic associated with growth traits through marker-assisted selection in most livestock such as in goats (Sarmah *et al*., 2020), chickens (Tyasi *et al*., 2018) and in cattle (Agung *et al*., 2018).

CONCLUSION

It can be concluded that *GH1* could be used as a genetic marker for marker-assisted selection (MAS) during goat breeding for excellent growth traits (withers height). The current study will assist farmers and breeders in selecting animals based on genetic markers to improve growth traits. Further investigation needs to be conducted on single nucleotide polymorphisms of the growth hormone gene and its association with the growth traits in larger sample size of Dorper sheep and more growth traits to be included.

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Conflict of interest

All authors declared that they have no conflict of interest.

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