ISSN 2664-4169 (Print) & ISSN 2664-7923 (Online)
South Asian Research Journal of Biology and Applied Biosciences
Abbreviated Key Title: South Asian Res J Bio Appl Biosci

| Volume-7 | Issue-3 | May-Jun -2025 | **DOI: https://doi.org/10.36346/sarjbab.2025.v07i03.008**

Original Research Article

Evaluation of Genetic Variation in the Calibage Gene in a Sample of Awassi Sheep

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Article History
Received: 01.05.2025
Accepted: 05.06.2025
Published: 11.06.2025

Abstract: This study was carried out to gain a better understanding of the genetic variability of the callipyge gene in the Awassi sheep breed and to offer useful data to researchers and breeders. The restriction fragment length polymorphism (RFLP) and polymerase chain reaction (PCR) techniques were used, and blood samples from seventy Awassi sheep were examined. To ascertain the genetic variety in the gene linked to the calibage phenomenon, the resulting genotypes were examined. The findings demonstrated that a single genotype, known as "GG," was found in every sheep sample that was part of the investigation. Indicating a decrease in genetic diversity for this gene in this strain.

Keywords: Genetic Variability, Genetic Diversity, Genotype, Polymorphism / Monomorphism, Allele.

INTRODUCTION

A gene named "callipyge," which means "beautiful buttocks" in Greek, was found by researchers from the U.S. Department of Agriculture and Duke University Medical Center [1]. This gene is responsible for the sheep's large, muscular bottoms and low fat content [2]. Because of this characteristic, which allows them to convert food into muscle 30% more efficiently than typical sheep, breeding these sheep may benefit [3]. A single nucleotide polymorphism (SNP) in the ovine DLK1-DIO3 imprinted domain on chromosome 18 is the cause of the callipyge phenotype [4]. At position 267 in the gene sequence, this SNP causes an adenine (A) to change to a guanine (G). A distinct inheritance pattern called polar overdominance is displayed by the callipyge mutation [5]. Thus, Polar Dominance Only in cases where the mutation is inherited from the sire (father) rather than the dam (mother) does the phenotype manifest. If the mutant allele is inherited from the father, heterozygous people (one mutant and one normal allele) display the callipyge phenotype; on the other hand, homozygous people (two mutant alleles) and heterozygotes inherited from the mother do not display the enhanced muscle development [6]. The callipyge mutation causes sheep to exhibit increased muscle hypertrophy, especially in the leg and loin muscles, but not as much fat deposition. Because of their higher lean meat yield, this makes them desirable for the production of meat. There may be disadvantages, though, such as tougher meat texture as a result of the higher muscle density [7]. Studies on the callipyge polymorphism have shed light on the regulation of growth and muscle development in mammals. Comprehending this genetic mutation holds potential benefits for livestock breeding initiatives that seek to enhance the productivity of meat production [8]. Although a lot of genetic research has been done on Iraqi sheep and goats, very little has been done on this particular gene [9-11], and the polymorphisms within the callipyge region have not received much attention from researchers worldwide. To that end, the current study's objectives were: Using the RFLP technique, describe the genotype frequency in the Iraqi sheep breeds Awassi and Arabi sheep.

MATERIAL AND METHODS

Awassi understudied Iraqi sheep breed, were represented by a total of 70 animals, which were randomly sampled. The strains came from Babylonian-raised herds. Blood samples were taken and brought to the lab in cooled conditions in tubes containing EDTA as an anticoagulant. Then, following the Al-Shuhaib (2017) [12], approach, genomic DNA was

extracted, put onto 1.5% agarose to identify the DNA bands, and stored at -20 °C until genetic tests were initiated. According to PCR conditions, 3µL of primer and genomic DNA were combined with the following elements: Ten milligrams.

Tris–HCl (pH 9.0), 1.5 mM MgCl2, 1 U Top DNA polymerase, 250 µM of each dNTP (dATP, dGTP, dTTP, and dCTP), and 30 mM KCl (BioNeer Company, Korea). The DNA fragment (Table 1) was amplified using a single primer and prepared by Sentibiolab Company, Turkey.

Table 1: The Identification of the Primers and its size

Gene	The primer sequences	Size
Callipyge	5'-TGA AAA CGT GAA CCC AGA AGC-3' 5'-GTC CTA AAT AGG TCC TCT CG-3'.	246 bp

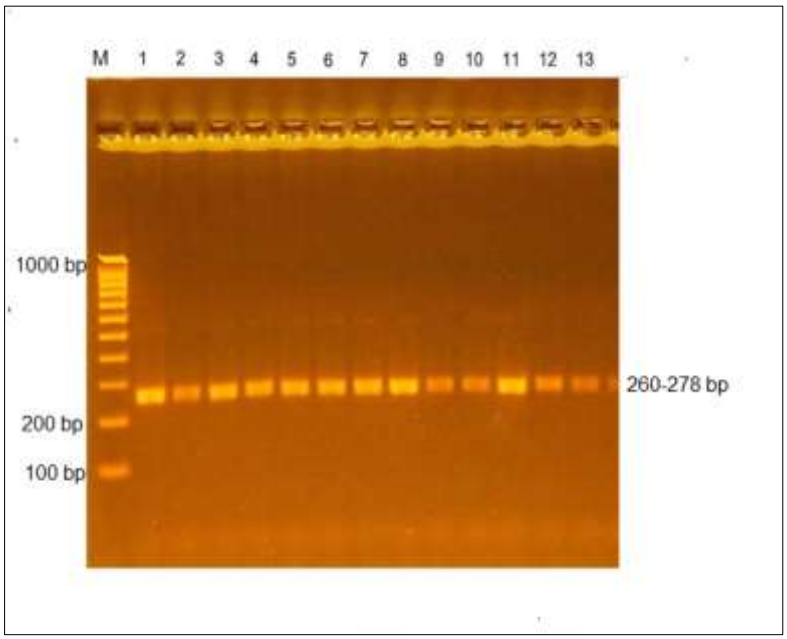
Initial denaturation, denaturation, annealing, extension, and lastly, final extension comprised the thermal profiling process for callipyge gene amplification. PCR procedure with BLG gene amplification. Procedures Procedure Temperature (°C) Time frame First Denaturation: 95-10 minutes 2 30 seconds of denaturation 94 62 30 seconds of 3 Annealing 4 Extension: 72–60 seconds 5 Last extension: 72 minutes. The Restriction Fragment Length Polymorphism (RFLP) technique was then utilized to identify genotyping employing a specific restriction enzyme that shows a pattern difference between the DNA fragment sizes for each organism (Bhattacharya *et al*., 2008). In order to evaluate the variation in DNA fragment size among Awassi sheep, the RsaI restriction enzyme (2U µL-1) was used in the current study for hours at 38 °C. The genotype and frequency of callipyge alleles in Awassi ewes were assessed using 2% gel agarose gel electrophoreses and restriction enzymes to break down the DNA product.

Statistical Analysis

SAS program (SAS, 2012) [13], was used to detect the results. The data was analysed using Completely Randomized Design (CRD).

Yij = µ +αi +eij Where: Where: µ: is an overall means, αi: the genotype of the prolactin gene. eij: is a randomly error, and genes (AA, AB, and BB) and their effect and eij: is an unexpected error. Estimates were done using the genotype and allele frequencies form [15]. Gene frequency =2D + H /2N If H is the number of animals with heterozygosis, D is the number of homozygous animals for specific alleles N: the overall number of animals. Chi-square (χ) test was used to indicate the important variations between individuals X = 2 Σ(Observed No. – Expected No.)2/Expected No. GLM, a general linear model, was uses in the study, and Duncan's numerous ranges Test13. To estimate the significant variance between groups

RESULT AND DISCUSSION



Figer 1: Agarose gel electrophoresis of PRL-PCR fragment (196 bp). Lane M, 100 bp DNA, lanes 1-13 Genotype GG(260-278) bp

Table 2: Genetic variation and frequency of alleles for the callipyge gene

Genotype	Number	Percentage(%)
TT :Wild	0	0.00
TG :Hetro	0	0.00
GG :Mutant	50	100.00
Total	50	% 100
(χ^2)	---	** 150.00
Allele frequency		
T	0.00	
G	(%100) 1	
**P<0.01.		

The results of treating the PCR products with restriction enzymes and putting them through gel electrophoresis after using the PCR-RFLP (Polymerase Chain Reaction - Restriction Fragment Length Polymorphism) technique on blood samples from 70 Awassi sheep showed the presence of a single genetic pattern, appointed as "GG".

The callipyge phenomenon, which involves a rise in muscular weight and can cause problems with the loin and hindquarters. The mutation resulting from this gene is passed down through an unusual method of inheritance known as polar overdominance, which determines its effect on the parent inheriting it. If the mutation is passed down through paternal, this outcome is only observed. As we can see in the results of this research, this sample of Awassi sheep has only one genetic pattern for this gene. This is consistent with Study [16], its conclusion was that the clpg locus is monomorphic for sheep populations in Saudi Arabia. The genotype AA with a frequency of 1.00 was identified. Additionally, in a another study of sheep from valachian, east friesian and lacaune breeds, the gene was only present in one genotype aa while other genotypes were absent [17].

CONCLUSIONS

Using restriction fragment length polymorphism (RFLP) and the polymerase chain reaction (PCR), blood samples from seventy Awassi sheep were examined. The genetic variety in the gene linked to the calibage phenomena was discovered by analyzing the resultant genotypes. The findings demonstrated that all of the sheep samples used in the investigation had the same genotype, which was called "GG."

REFERENCES

1. Nabokov, V.V., 1961. Nikolai Gogol (No. 78). New Directions Publishing.
2. Cockett, N.E., Jackson, S.P., Snowder, G.D., Shay, T.L., Berghmans, S., Beever, J.E., Carpenter, C. and Georges, M., 1999. The callipyge phenomenon: evidence for unusual genetic inheritance. *Journal of Animal Science*, *77*(suppl_2), pp.221-227.
3. Georges, M., Charlier, C. and Cockett, N., 2003. The callipyge locus: evidence for the trans interaction of reciprocally imprinted genes. *Trends in Genetics*, *19*(5), pp.248-252.
4. Hagan, J.P., O'Neill, B.L., Stewart, C.L., Kozlov, S.V. and Croce, C.M., 2009. At least ten genes define the imprinted Dlk1-Dio3 cluster on mouse chromosome 12qF1. *PloS one*, *4*(2), p.e4352.
5. Freking, B.A., Murphy, S.K., Wylie, A.A., Rhodes, S.J., Keele, J.W., Leymaster, K.A., Jirtle, R.L. and Smith, T.P., 2002. Identification of the single base change causing the callipyge muscle hypertrophy phenotype, the only known example of polar overdominance in mammals. *Genome research*, *12*(10), pp.1496-1506.
6. Cockett, N.E., Jackson, S.P., Shay, T.L., Farnir, F., Berghmans, S., Snowder, G.D., Nielsen, D.M. and Georges, M., 1996. Polar overdominance at the ovine callipyge locus. *Science*, *273*(5272), pp.236-238.
7. Freking, B.A., Smith, T.P.L. and Leymaster, K.A., 2004. The Callipyge mutation for sheep muscular hypertrophy-genetics, physiology and meat quality. In *Muscle development of livestock animals: Physiology, genetics and meat quality* (pp. 317-342). Wallingford UK: CABI Publishing.
8. Nguyen, T., 2009. *Links between the IGF-1 signalling pathways and skeletal muscle hypertrophy in sheep*. University of Western Australia.
9. Zaidan, H.H. and Al-Sarai, T.M., 2024, July. The Impact of Biological and Chemical Fungicides on Sheep Lymphocytes' Ability to Induce Chromosomal Aberration. In *IOP Conference Series: Earth and Environmental Science* (Vol. 1371, No. 7, p. 072050). IOP Publishing.
10. Tamarah M. Alsarai., 2022. Effect of the prolactin gene polymorphism on quantity and quality of milk in Iraqi goats. *Bionatura journal, 7*(4), pp.1-4.
11. Dakheel, M.H. and al-Anbari, N.N., Effect of IGF2 Polymorphism on Body Weight and Body Dimensions of Fallow Deer in Iraq
12. SAHIB A. AL-SHUHAIB, M.B., 2017. A universal, rapid, and inexpensive method for genomic DNA isolation from the whole blood of mammals and birds. *Journal of Genetics*, *96*(1), pp.171-176.

13. SAS 2012, User's Guide. Statistical. Statistical Analysis System Version 9.1th ed. SAS. Institute Inc. Cary, NC, USA
14. Khudair, T.Y. and Albbas, F.A.A., 2021. Effect of silicon and humic acid on vegetative and flowering growth traits in gazania plant (Gazania splendens). *International Journal of Agricultural and Statistical Sciences*, *17*(1), pp.73-80.
15. Falconer DS and Mackay TFC. Introduction to Quantitative Genetics, (4th Ed.). Longman Group Limited, England, 1996.
16. Alakilli, S.Y., 2015. Analysis of polymorphism of caplstatin and callipyge genes in Saudi sheep breeds using PCR-RFLP technique. *International Journal of Pharmaceutical Sciences Review and Research*, *30*(1), pp.340-344.
17. Selvaggi, M.A.R.I.A., Laudadio, V., Dario, C. and Tufarelli, V.I.N.C.E.N.Z.O., 2015. β-Lactoglobulin gene polymorphisms in sheep and effects on milk production traits: a review.