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Polymorphism of the callipyge gene in the Esme sheep breed

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Abstract

Mutation of the candidate gene, callipyge (*CLPG*), causes muscular hypertrophy, primarily in the pelvic and hind limbs, and therefore has a major impact on sheep development and quality. Finding the *CLPG* gene polymorphism in the Esme sheep breed was the study's main goal. DNA from 50 Esme sheep blood samples was used as the study material in this investigation. The CLPG/FaqI polymorphism was examined in all sheep using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique, following which, only the A allele and AA genotype were found. The results of this study indicate that the CLPG/Faq1 polymorphism in the Esme breed is monomorphic. This study is the first to examine the *CLPG* gene polymorphism in the breed.

Keywords: *CLPG* gene, callipyge gene, polymorphism, restriction fragment length polymorphism
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Introduction

Sheep are an important livestock species and provide people with milk, meat, and textile fibre for everyday use (Li *et al*., 2022). Numerous genetic and environmental factors influence these quantitative features (Bao *et al*., 2021). Understanding how a trait originates requires an explanation of the genetic and epigenetic factors underlying these economic features (Houschyar *et al*., 2020). Thanks to advances in sequencing technologies and biotechnology, it is now easier to find functional genes that improve the financial aspects of sheep and goat production (Li *et al*., 2022).

Mutton is one type of health food that is abundant in nutrients like protein and low in fat and cholesterol. The rapid expansion of the meat sheep business in our nation is substantially supported by the rise in the consumption of sheep meat throughout time, as well as the ongoing improvements in per capita income and the growing awareness of leading a healthy lifestyle (Li and Jin, 2019; Wang *et al*, 2022). Quantitative qualities are the only ones that can be improved by traditional genetic improvement breeding techniques to increase animal productivity and carcass quality.

Modern DNA technology tools enable the identification of genes directly or indirectly linked to features that are commercially important and new methods for evaluating animals are part of this technology (Gorlov *et al.*, 2014; Zinovieva *et al.*, 2015; Yaşar *et al.*, 2024). Breeding and genomic selection at the DNA level can be carried out in addition to conventional animal selection if selection-preferred gene variants are identified (Deniskova *et al*., 2017). The ability to identify genes that impact economically-significant features and use them in genomic selection research is made possible by advancements in molecular technology. Molecular research is essential to animal breeding in this sense.

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Muscle hypertrophy can result from a mutation in the callipiyge gene (*CLPG*), a functional gene that was initially discovered in the 1983 breeding of Dorset sheep. This gene influences the development and softness of animal muscles (Jawasreh *et al*., 2019). Only one nucleotide is altered in the *CLPG* mutation (G-A). Because the *CLPG* gene increases carcass weight while maintaining the appropriate level of fat in the carcass, it is crucial in sheep breeding. According to a number of studies (Jawasreh *et al*., 2016; Penick *et al*., 2017), lamb carcasses with the *CLPG* mutation are more valuable and sought after than lamb carcasses without the mutation. Little research has been done in Turkey on the *CLPG* gene, despite the fact that it has a major impact on the quality of meat (Kavuzkoz & Kırıkçı, 2023). There has been no published research on the genotypic organization of *CLPG* for the Esme breed. The genetic makeup of *CLPG* in Esme sheep is reported for the first time in this study.

The majority of molecular studies on meat yield in Turkish sheep breeds have focused on gypsum leptin and a few other genes, with less attention paid to the *CLPG* gene. Furthermore, no research has been done to date to explain the genetic makeup of the *CLPG* gene in the Esme sheep breed. A growing number of Turkish native and hybrid breeds are becoming interested in the *CLPG* gene for its potential in the improvement of growth and composition of lamb carcasses.

Material and Methods

Ethical approval and permission for this study was obtained from Uşak University Animal Experiments Local Ethics Committee (Date: 03/07/2024; Decision No: 2024/01).

DNA samples were taken in blood from 50 Esme sheep, regardless of gender and age, on a farm in the Esme district, Turkey. A commercial DNA isolation kit was used to isolate the DNA. For the amplification of the *CLPG* gene, polymerase chain reaction (PCR) was performed with primer pairs (F, 5'-TGAAAACGTGAACCCAGAAGC-3'; R, 5'-GTCCTAAATAGGTCCTCTCG-3'). Reaction conditions for PCR were created in a final volume of 25 µL, containing 10 µL of Master Mix red (2X), 0.7 µL of both forward and reverse primers, 3 µL of pure DNA, and 6.3 µL of H_2O. The following conditions were used for the PCR: a 5-min initial step at 95 °C, 35 cycles of denaturation at 95 °C for 35 s, annealing at 56 °C for 30 s, extension at 72 °C for 1 min, and a final extension at 72°C for 5 min. The 426-bp length PCR products were digested using the FaqI restriction enzyme in preparation for the restriction fragment length polymorphism (RFLP) method. A final reaction volume of 30 µL, comprising 1.2 µL of fast digest FaqI, 18 µL of distilled water, 2 µL of 10X Tango buffer, 6 µL of 50X SAM, and 10 µL of the enzyme from the PCR product was used for the digestion process. After 16 h of incubation at 37 °C and 20 min at 80 °C, the RFLP reaction mixture was used to identify the genotypes of the animals. Safe-Green dye was used to view the samples following RFLP analysis, which was followed by 3% high-resolution agarose gel electrophoresis. In order to identify potential genotypes in the Esme breed, a 426-bp segment of the *CLPG* gene was amplified using PCR. The PCR products were subsequently digested using the FaqI restriction enzyme. Following PCR-RFLP, the results were run through 3% agarose gel electrophoresis (Figure 1).

Results

Two fragments (395 bp and 31 bp) for the mutant allele G and three fragments (278 bp, 117 bp, and 31 bp) for the mutant allele A were obtained. The results demonstrated that every individual either carried the mutation (A → G transition) or did not possess the wild-type genotype (Figure 1).

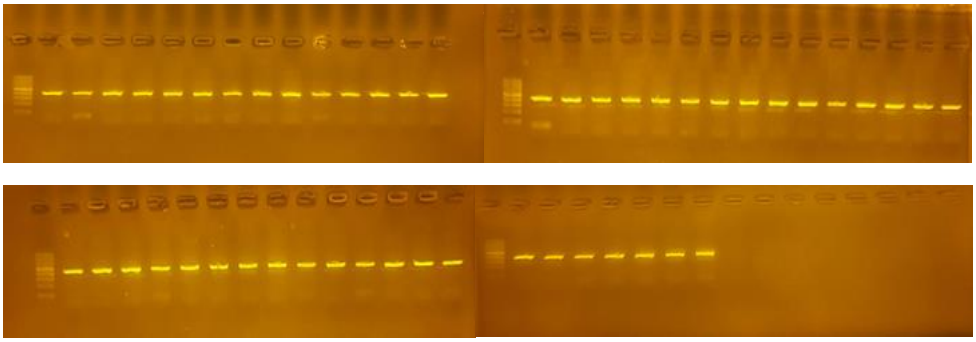

Figure 1. Findings from polymerase chain reaction-restriction fragment length polymorphism amplification of the 426-kb *CLPG* gene

The genotype of every sheep was found to be AA. As a result, the study's population frequency for the AA genotype was 1.00, making it impossible to evaluate genetic balance. Since the A allele only detected one allele, allele frequencies were not compared. The polymorphism of the *CLPG* gene in Esme sheep has not yet been studied. First-hand results have been acquired with reference to this breed. The allelic variants and genotypes of the *CLPG* gene were discovered using molecular genetic research done on sheep of the Esme breed. In these sheep, only the homozygous AA genotype was found (Figure 2 A–C). The genotypes of AG and GG are unknown. These sheep had a monomorphic *CLPG* locus.

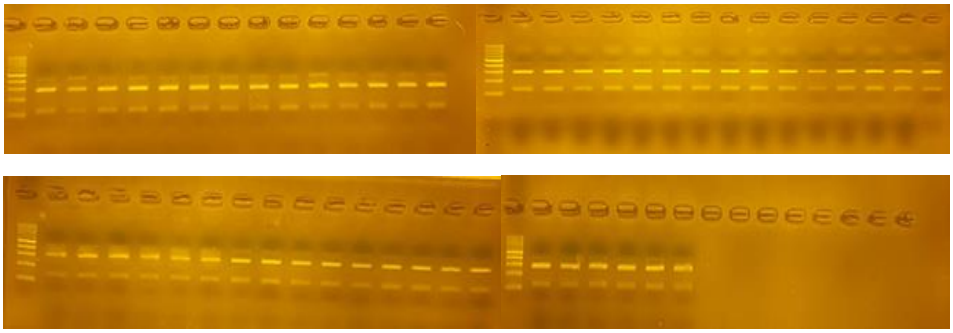

Figure 2. Electropherograms and AA genotypes of the polymerase chain reaction-restriction fragment length polymorphism result of the *CLPG*/Faq1 gene in 3% agarose gel

Discussion and Conclusion

The results indicated that the Esme sheep under study did not possess the mutant G allele for CLPG. Consequently, A and AA had the highest genotype and allele frequencies, respectively, and this indicates that Esme sheep are monomorphic at the *CLPG* locus. Comparing allele frequencies was not possible because the study only found one allele. Furthermore, the χ^2 test was not used to verify the Hardy–Weinberg balance because the frequency of the AA genotype was 1.00.

Only the AA genotype was found in Iranian sheep of the Lori breed in the study of Nanekarani *et al*. (2014) study, indicating that the sheep were monomorphic at the *CLPG* gene. Furthermore, Alakilli (2015) reported that the CLPG locus was monomorphic for the population of sheep bred in Saudi Arabia. This was also confirmed by Dimitrova & Bozhilova-Sakova (2016), who found genetic variation in *CLPG* in sheep of the Karakachan breed. The CLPG locus was found to be monomorphic in the current group, with only the homozygous AA genotype. According to research on *CLPG* gene polymorphisms, Russian sheep breeds such Kalmyk, Volgograd, and Edilbay have homozygous AA genotypes for CLPG allelic variations and a monomorphic CLPG locus (Gorlov *et al*., 2020). With only the AA genotype, similar outcomes were noted in the Saudi (Alakilli, 2015), Karakachan (Dimitrova & Bozhilova-Sakova, 2016), and Persian Lori (Nanekarani & Goodarzi, 2014) breeds. These results are similar to the findings of the current study.

The gene can be utilized as a marker for sheep meat production, as demonstrated by Jackson *et al*. (1997). The *CLPG* gene is one of the genes potentially influencing meat output. Numerous scientists advise using the *CLPG* gene as a DNA marker to influence greater meat yield because it aids in the intense development of muscle tissue (Busboom *et al*., 1999). Due to the mutation, certain muscle groups in the thighs of CLPG sheep have hypertrophy and a minimal amount of fat (Selionova *et al*, 2017). According to reports, callipyge mutations containing 25% of the Rambouillet gene can effectively increase the development and meat qualities of Awassi sheep, with the exception of tenderness (Freking *et al*, 2018).

According to Jackson *et al*. (1997), CLPG lambs have lower daily feed consumption and improved feed efficiency, which lowers production costs. Consumer lamb prices might be lowered, and the sheep business could become more profitable by using the *CLPG* mutation in introgression experiments in domestic sheep breeds (Esen *et al*., 2022a, b). With the exception of a few breeds, very little research has been done on the *CLPG* gene in domestic sheep breeds in Turkey. The results of this study are the first for the Esme sheep breed in particular.

The fact that domestic Turkish sheep breeds carry the gene but lack the CLPG phenotype may account for the paucity of studies on CLPG in general. However, the current study's initial findings have clarified the molecular makeup of the Esme breed's genetic makeup.

The origin of the Esme sheep, whose homeland is the Uşak region, is the Dağlıç breed. Over time, the characteristics of the breed began to emerge as a result of crossbreeding with Kıvırcık, and later with Sakız sheep, making it a genotype with combined productivity (Anonymous, 2014). A sizable portion of Turkey's sheep population are mixed-breed Esme sheep (Ünal, 2002). Because of this, the Esme sheep breed may be less successful than other local breeds in producing a genetic mutation that can be utilized to enhance the quality of the meat. Determining if genetic selection studies have been conducted is challenging (Ceyhan *et al*., 2019). Comprehensive research is needed to investigate potential or additional genes influencing the meat yield and quality of domestic sheep breeds because traditional breeding techniques are labour- and time-intensive. According to Gootwine *et al*. (2008) and Jawasreh *et al*. (2019), breeding research has suggested a method of enhancing meat yield and quality by introducing the CLPG mutation to non-mutant races. Awassi with a mutation in the *CLPG* gene crossed with Rambouillet significantly improved carcass quality and growth, according to Jawasreh *et al*. (2019).

This is the first study to look into the *CLPG* gene of the Esme breed. More thorough research could yield more accurate details regarding the genetic makeup of the *CLPG* gene in the Esme breed. The findings broaden our understanding of the molecular markers that define the quality of sheep meat.

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Conflicts of interest

The author declares no conflict of interest for this article.

Availability of data and material

Data will be available on request.

References

Alakilli, S.Y., 2015. Analysis of polymorphism of caplstatin and callipyge genes in Saudi sheep breeds using PCR-RFLP technique. Int J Pharm Sci Rev Res. 30, 340–344.

Bao, G., Liu, X., Wang, J., Hu, J., Shi, B., Li, S., Luo, Y., 2021. Effects of slaughter age on myosin heavy chain isoforms, muscle fibres, fatty acids, and meat quality in longissimus thoracis muscle of Tibetan sheep. Front Vet Sci. 8. doi.org/10.3389/fvets.2021.689589

Busboom, J.R., Wahl, T.I., Snowder, G.D., 1999. Economics of callipyge lamb production. J Anim Sci. 77, 243–248.

Ceyhan, A., Şekeroğlu, A., Duman, M., 2019. Some reproductive traits and lamb growth performance of Akkaraman sheep raised in Niğde province. Turk Tarim Gida Bilim Teknol Der. 7(10), 1509–1514.

Deniskova, T., Dotsev, A.V., Selionova, M., Wimmers, K., Reyer, H., Kharzinova, V.R., Brem, G., Zinovieva, N.A., 2017. Whole-genome single nucleotide polymorphism study of Romanov sheep. J Anim Sci. 95, 339–340.

Dimitrova, I., Bozhilova-Sakova, M., 2016. PCR-RFLP analysis of callipyge gene (*CLPG*) in Karakachan sheep breed. Bulg. J. Agric. Sci. 22, 482–484.

Esen, V.K., Esen, S., Karadağ, O., Önenç, A., Elmacı, C., 2022a. Genotypic characterization of meat-type lambs expressing the callipyge gene in Turkey: I. Carcass characteristics and retail yield. Turk J Vet Anim Sci. 46(1), 157–164.

Esen, V.K., Esen, S., Karadağ, O., Elmacı, C., 2022b. Genotypic characterization of meat-type lambs expressing the callipyge gene in Turkey: II. Effect on body indexes. Small Rumin. Res. 208, 106633.

Freking, B.A., King, D.A., Shackelford, S.D., Wheeler, T.L., Smith, T.P.L., 2018. Effects and interactions of myostatin and callipyge mutations: I. Growth and carcass traits. J Anim Sci. 96, 454–461.

Gootwine, E., Reicher, S. and Rosov, A., 2008. Prolificacy and lamb survival at birth in Awassi and Assaf sheep carrying the *FecB* (Booroola) mutation. Anim Reprod Sci. 108, 402–411.

Gorlov, I.F., Fedunin, A.A., Randelin, D.A., Sulimova, G.E., 2014. Polymorphisms of *bGH*, *RORC*, and *DGAT1* genes in Russian beef cattle breeds. Russ. J. Genet. 50 1302–7.

Gorlov, I.F., Shirokova, N.V., Kolosov, Y.A., Kolosov, A.Y., Getmantseva, L.V., Slozhenkina, M.I., Mosolova, N.I., Anisimova, E.Y., Ponomariov, V.V., 2020. Polymorphism of *CLPG* gene in three sheep breeds grown in the steppe zone of the Russian Federation. J. Adv. Vet. Anim. Res. 7, 51–55.

Houschyar, K.S., Borrelli, M.R., Tapking, C., Popp, D., Puladi, B., Ooms, M., *et al*., 2020. Molecular mechanisms of hair growth and regeneration: Current understanding and novel paradigms. Dermatol. 236, 271–280.

Jackson, S., Green, R., Miller, M., 1997. Phenotypic characterization of Rambouillet sheep expressing the callipyge gene: I. Inheritance of the condition and production characteristics. J Anim Sci. 75, 14–18.

Jawasreh, K.I., Al-Amareen, A.H., Abdullah, A.Y., 2016. Growth performance and meat characteristics of Awassi sheep that hold the callipyge gene. J Anim Sci. 94(4), 175–176.

Kavuzkoz, M., Kırıkçı, K., 2023. Investigation of callipyge gene polymorphism in Akkaraman sheep breed. Yüzüncü Yıl Üniversitesi Fen Bilimleri Enstitüsü Dergisi. 1, 215–219.

Li, J., Jin, H., 2019. Development overview, future trends, and countermeasures of meat sheep and goat industry in 2019. Chinese J Anim Sci. 55, 138–145.

Li, S., Wang, X., Su, R., 2022. Editorial: Sheep and Goat Gene Exploration, Front Gene. 13, 802709.
Nanekarani, S., Goodarzi, M., Mahdavi, M., 2014. Analysis of polymorphism of callipyge gene in Lori sheep by PCR-RFLP Method. APCBEE Procedia. 8, 65–69.
Nanekarani, S. & Goodarzi, M., 2014. Polymorphism of candidate genes for meat production in Lori sheep. IERI Procedia. 8, 18–23.
Penick, M., Kim, H. W., Setyabrata, D., Waddell, J. N., Bidwell, C. A., & Kim, Y. H. B., 2017. Callipyge genotypic effects on meat quality attributes and oxidation stability of ovine *M. longissimus*. Small Rumin Res. 146, 5–12. doi:10.1016/j.smallrumres.2016.11.011
Selionova, M.I., Skorykh, L.N., Fominova, I.O., Safonova, N.S., 2017. Genome selection in sheep breeding. Proceedings of the All-Russian Research Institute of Sheep and Goat Breeding, Russia, Stavropol, 275–280.
Ünal, N., 2002. Survival rate, growth characteristics, and some body measurements of Akkaraman and Sakız (Chios) × Akkaraman F1 lambs. Turk J Vet Anim Sci. 26(1), 109–116.
Wang, J.T., Wang, G.S., Gong, Y.F., Qiao, X., Li, X., Wang, G.Z., Zheng, Y.Z., Lv, J.G., Li, X.L., Liu, Z.Z., 2022. Screening of three-way crossbred combination and genetic effect analysis of the SNP in the *CLPG* gene in meat sheep. Arch Anim Breed. 65, 417–426.
Yaşar, Ü., Ayvazoğlu, C., Yasar, Z.G., Kızıltepe, Ş., Aydın, N., 2024. High-sensitivity cardiac troponin (hs-cTnI and hs-cTnT) levels in dogs with hepatozoon canis. ISPEC J Agric Sci. 8(2), 482–492.
Zinovieva, N.A., Selionova, M.I., Gladyr, E.A., Petrovic, M.P., Caro, P.V., Ruzic, M.D., *et al*., 2015. Investigation of gene pool and genealogical links between sheep breeds of southern Russia by blood groups and DNA microsatellites. Genetika-Belgrade. 47, 395–404.