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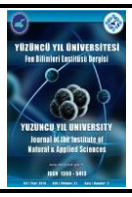

Research Article

Investigation of Callipyge Gene Polymorphism in Akkaraman Sheep Breed

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Abstract: *Callipyge* (*CLPG*) is a candidate gene with a significant effect on meat quality and growth in sheep. The aim of this study was to investigate the polymorphism of the *CLPG* gene in the Akkaraman breed. In the study, DNAs obtained from the blood samples of 50 Akkaraman sheep was used as study material. The *CLPG*/*Faq*1 polymorphism was examined in all individuals using the method PCR-RFLP. Only A allele and AA genotype were detected after PCR-RFLP procedure. This is the first study in which *CLPG* gene polymorphism was investigated in Akkaraman breed, and the findings showed that the Akkaraman breed has a monomorphic structure in terms of *CLPG*/*Faq*I polymorphism.

Akkaraman Koyun Irkında Callipyge Gen Polimorfizmin Araştırılması

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Öz: *Callipyge* (*CLPG*) koyunlarda et kalitesi ve büyüme üzerinde önemli etkisi olan bir aday gendir. Çalışmada Akkaraman koyun ırkında *CLPG* gen polimorfizmin belirlemesi amaçlanmıştır. Araştırmada Akkaraman ırkı 50 baş koyuna ait kan örneklerinden elde edilen DNA'lar çalışma materyali olarak kullanılmıştır. Tüm bireylerde *CLPG*/*Faq*I polimorfizmi PCR-RLP yöntemi ile araştırılmıştır. PCR-RFLP işlemi sonrasında sadece A alleli ve AA genotipi tespit edilmiştir. Bu çalışma *CLPG* gen polimorfizmin Akkaraman ırkında araştırıldığı ilk çalışma olup elde edilen bulgular Akkaraman ırkının *CLP*/*Faq*1 polimorfizmi bakımından monomorfik bir yapıya sahip olduğunu göstermiştir.

1. Introduction

The most of economically valuable features in animal breeding are quantitative characters which are shaped by polygenic inheritance and environmental factors. The genetic improvement by traditional breeding methods is restricted for quantitative traits. Scientists can identify genes that impact economically relevant characteristics and include them in genomic selection studies, thanks to advancements in molecular technology (Cesarani et al., 2019; Gorlov et al., 2020; Mohammed et al., 2022). In this regard, molecular studies play a critical role in animal breeding.

The performance of growth and development within a certain period is a critical aspect determining profit in sheep farming. To decide on genetic breeding strategies, it is important to know the genetic potential of domestic sheep breeds in terms of traits that are important to industry, such as meat quality and yield.

The *callipiyge* gene (*CLPG*), first described in 1983 in the Dorset breed of sheep, is one of the well-studied genes affecting sheep muscle development and is located on sheep chromosome 18 (Cockett et al., 1996). The *CLPG* mutation occurs with a single base change (G-A) that does not disrupt the protein coding sequence (Jackson et al., 1997). The *CLPG* causes muscle hypertrophy that is restricted to the pelvic and lumbar muscles with little or no effect on the anterior skeletal muscles (Cockett et al., 1996). As described in several studies, this gene also provides a higher leg score and carcass percentage, larger longissimus loins, superior leanness composition, higher leg values, higher percentage of dressing percentages, and optimal feed conversion, as well as higher body weight and Baron-Crevat indices (Jackson et al., 1997; Jawasreh et al., 2019; Esen et al., 2022b).

Most of the molecular studies on meat yield in Turkish sheep breeds have been performed on the cast, leptin, and a few other genes, while the *CLPG* gene was studied less (Balcıoğlu et al., 2014; Bayram et al., 2019; Kırıkçı et al., 2021; Kırıkçı, 2022). In addition, there is currently no study that describes the genetic structure of the Akkaraman sheep breed for the *CLPG* gene. Nonetheless, there is a growing interest in the *CLPG* gene in several native and cross breeds in Turkey (Esen et al., 2022a and 2022b). Researchers reported that the Kıvırcık breed, an indigenous Turkish sheep breed, has the *CLPG* mutation. Considering the previous studies, the aim of this study was to determine the genetic structure of the *CLPG* gene in the Akkaraman breed with the highest number within the sheep population in Turkey

2. Material and Methods

DNA samples were extracted from blood samples of 50 sheep of the Akkaraman breed collected from seven flocks as part of two subprojects (TAGEM/66 AKK2011-01 and AKK2012-02) in the Yozgat province (Ethical permission number: 2021/3, Ahi Evran University, Krşehir, Turkey). DNA isolation was performed using a commercial DNA isolation kit (Genomic DNA isolation kit, Thermo Scientific,). PCR process was aplplied with primer pairs (forward; 5' TGAAAACGTGAACCCAGA AGC3', reverse; 5'GTCCTAAATAGGTCCTCTCG3') for the amplification of *CLPG* gene. PCR was performed under reaction conditions prepered in 25 µL final volume, including13 µL Master Mix red (2X) (1.5 mM of $MgCl_2$ in final concentration), 1µL forward and reverse primer, 1.5µL pure DNA and 9.5µL H_2O. The PCR conditions were performed as follows: and first 95°C for 5 min, followed by 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.

PCR products of 426 bp length were digested with the FaqI restriction enzyme for the RFLP process. Degistion was performed in a final volume of 30 µL consisting of 1.2 µL of the fast digest *Faq*I enzyme (Thermo Scientific, ER1811) 10 µL of the PCR product, 2 µL of 10X buffer Tango, 0.6 µL of 50X SAM and 18 µL pure water. To determine the animals' genotypes, reaciton mixture for RFLP was incubated at 37 °C for 16 h and then at 80 °C for 20 min. After RFLP analysis, the samples were run in 3% high resulation agarose gel electrophoresis and then visualized with green safe dye.

3. Results

In the present study, a part of 426 bp of the *CLPG* gene was amplified by PCR and the PCR products were then subjected to digestion of the restriction enzyme *Faq*I to identify possible genotypes in the Akkaraman breed. After PCR-RFLP, the products were electrophoresed in a 3% agorose gel (Figure 1). Two fragments of 395 bp and 31 bp were expected for the mutant allele G, while three fragments of 278 bp, 117 bp and 31 bp were expected for allele A. The results showed that all individuals had a wild type genotype or carried no mutation (A → G transition), as shown in Figure 1.

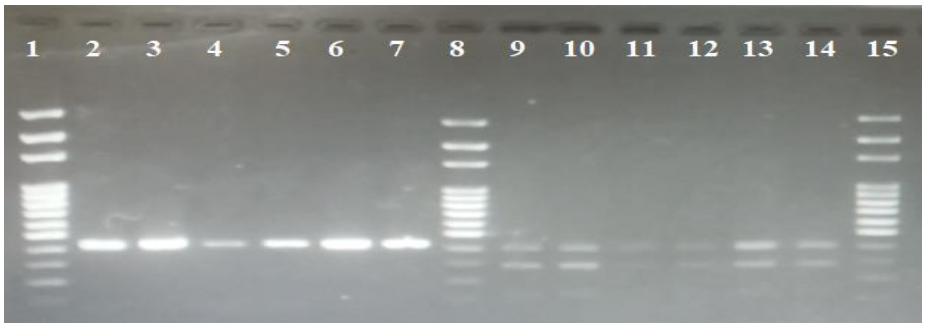

Figure 1. The results of 426 bp *CLPG* gene amplification with the PCR-RFLP. Line: 1,8,15; 100 bp DNA ladder, Line 2-7; PCR results and Line 9-14; AA genotypes.

All ewes were in the AA genotype. Therefore, the frequency of the AA genotype in the study population was 1.00, which did not lead to an assessment of genetic equilibrium. Also, allele frequencies were not compared with each other due to detecting only one allele, A.

4. Discussion and Conclusion

The *CLPG* gene plays an important role in sheep breeding because it ensures a higher carcass weight with the desired fat content of the carcass. Several studies have shown that the carcasses of lambs carrying a *CLPG* mutation are more desirable and profitable than those without the mutation (Jawasreh et al., 2016; Penick et al., 2017). Despite *CLPG* gene's important influence on meat quality, there have been few studies on the *CLPG* gene in Turkey. On the other hand, no studies of the genotypic structure of *CLPG* have been published for the Akkaraman breed.

The present study provided first report about the genetic structure of *CLPG* in Akkaraman sheep. The findings demonstrated that there was no mutant G allele for the *CLPG* in the studied Akkaraman sheep. Therefore, the highest allele and genotype frequencies were found to be A and AA, respectively. In the study, the allele frequencies were not compared with each other, as only one allele was detected. Besides, since the frequency of the AA genotype was 1.00, the Hardy-Weinberg equilibrium was not checked on the basis of the X^2 test. These results were similar to a study by Gabor, who reported that only the homozygous genotype AA for the *CLPG* gene occurred in sheep of the Tsigai, improved Valachian, East Friesian, Lacaune breeds and the Lacaune and Tsigai crosses. The same result was also found in North-Eastern Bulgarian Merino sheep (Bozhilova-Sakova et al., 2020), Lori sheep from Iran (Nanekarani et al., 2014), the Avicalin, Bharat Merino, Nellore, Chokla, and Mapura breeds from India (Meena et al., 2018). However, the results obtained from the current study were in contrast to those for the Dorset, Ramboillet and Hampshire sheep breeds (Jackson et al., 1997). One possible explanation for the presence of mutation in these breeds is that the *CLPG* mutation originated in the Dorset breed and spread to other breeds (Cockett et al., 1994). Esen et al. (2022a), who studied the structure of the *CLPG* gene in five sheep breeds, reported the genotype frequencies MN-12.50 and NN-93.75 for the Kıvırcık breed**.** Some studies have shown that the advantages of the *CLPG* mutation include larger longissimus loin eye areas, better lean composition, higher leg values and dressing percentages, and optimal feed conversion (Koohmaraie et al., 1995; Jackson et al., 1997). Jawasreh et al. (2019) discovered that the *CLPG* mutation, which contains 25% Rambouillet genes, can improve growth and meat quality.

CLPG lambs have higher feed efficiency and lower daily feed intake, resulting in lower production costs (Jackson et al., 1997). The use of the *CLPG* mutation in native sheep breeds in studies of introgression can lower the price of lambs for consumers and make the sheep industry more profitable (Esen et al., 2022a and 2022b). There were almost no studies on the *CLPG* gene in Turkish native sheep breeds except for a few breeds and the findings of the current study were the first for the Akkaraman breed, which is the most common sheep breed in Turkey. In general, the lack of research on the *CLPG* gene may be due to the absence of the *CLPG* phenotype in domestic Turkish sheep breeds. However, the preliminary results of this study have clarified the genetic structure of the Akkaraman breed at the moleculer level.

The Akkaraman sheep, which is a fat-tailed and combined breed, represents an important part of the Turkish sheep population (Ünal, 2002). Therefore, the detection of a mutation that can be utilized

to genetically improve meat quality in the Akkaraman sheep breed may have a higher impact than in other indigenous breeds with small numbers It is difficult to say whether or not genetic selection studies are performed for the Akkaraman breed because most selection studies are conducted by breeders based on morphological observations and previous experiences (Ceyhan et al., 2019). Considering that conventional breeding methods are difficult and time-consuming, possible or other genes affect meat yield and quality of domestic sheep breeds, especially the Akkaraman breed, should be investigated in comprehensive studies.

In breeding studies, the intogression of the *CLPG* mutation to non-mutant breeds has been suggested as a method for enhancing meat yield and quality (Gootwine et al., 2003; Jawasreh et al., 2019). According to the data obtained by Jawasreh et al. (2019), who crossbred Awassi with Rambouillet having the mutation, the *CLPG* gene significantly improved growth and carcass quality. In addition to crossbreeding, it is crucial for the implementation of marker-assisted selection within a breed that the related gene exhibits variation**.** Esen et al. (2022a) pointed out that *CLPG* gene could be adopted to selection programs thanks to the polymorphic structure in the most breeds they studied. However, the knowledges obtained from this study could be not used for the selection studies aiming to increase productivity in the studied Akkaraman ewes due to non-polymorphic structure of the studied gene. When the samples were collected from the Akkaraman breed, no lambs with the *CLPG* phenotype were observed. Even if the Akkraman breed did not carry the mutation, there was no evidence at the molecular level about the structure of the *CLPG* gene in the Akkraman breed. The present study was the first to investigate the *CLPG* gene in the Akkaraman breed. Studies with high sample sizes may yield more definitive information regarding the genetic structure of the *CLPG* gene in the Akkaraman breed.

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