OKU Fen Bilimleri Enstitüsü Dergisi
7(5): 2364-2373, 2024

OKU Journal of The Institute of Science and Technology, 7(5): 2364-2373, 2024

Osmaniye Korkut Ata Üniversitesi
Fen Bilimleri Enstitüsü
Dergisi

Osmaniye Korkut Ata University
Journal of The Institute of Science and Technology

Relationship of Calpastatin Gene Polymorphism with Growth Traits in Some Locally Reared Awassi and Akkaraman Sheep Breeds

Mehmet DOKGÖZ[1], Bahri Devrim ÖZCAN[2*]

[1]Çukurova University, Institute of Applied and Natural Sciences, Department of Animal Science, 01330, Adana, Türkiye
[2]Çukurova University, Faculty of Agriculture, Department of Animal Science, 01330, Adana, Türkiye

[1]https://orcid.org/0009-0002-9793-8013
[2]https://orcid.org/0000-0002-9198-656X
*Corresponding author: bdozcan@gmail.com

Research Article

Article History:
Received: 31.03.2024
Accepted: 08.07.2024
Published online:10.12.2024

Keywords:
CAST gene
Awassi sheep
Akkaraman sheep
Polymorphism
Genotype frequencies

ABSTRACT

In this study, it was aimed to determine the polymorphism of the Calpastatin gene (*CAST*) in Awassi and Akkaraman sheep which were reared in Osmaniye province within the scope of the National Sheep Breeding Project in the Hand of the Public. The study was carried out on a total of 39 blood samples taken from 20 head Awassi sheep and 19 head Akkaraman sheep reared in 5 different farms. In the study in which PCR-RFLP method was used, 622 bp long PCR amplicons obtained from genomic DNA samples of Awassi and Akkaraman sheep breeds for *CAST* gene were digested with *Msp*I restriction enzyme; Two bands of 336 and 286 bp in length for homozygous MM allele, a single band of 622 bp in length for homozygous NN allele and three bands of 622, 336 and 286 bp in length for heterozygous MN alleles were observed. The frequencies of M and N alleles in the Akkaraman breed were 0.82 and 0.18, respectively, while the frequencies of M and N in the Awassi breed were 0.75 and 0.25, respectively. The frequencies of MM, MN, and NN genotypes were 0.60, 0.30, and 0.10, respectively. Contrary to the Awassi breed, only MM and MN genotypes were observed in Akkaraman sheep and their frequencies were calculated as 0.63 and 0.37, respectively. While deviation in Hardy-Weinberg equilibrium was found to be insignificant in the Akkaraman population, it was found to be significant in Awassi sheep. The association analysis revealed that there was a significant association ($P<0.05$) between *CAST* gene polymorphism and 90th-day weight in Awassi breeds, while this association was not found in the Akkaraman breed ($P<0.05$).

Halk Elinde Yetiştirilen Bazı İvesi ve Akkaraman Koyun Irklarında Calpastatin Gen Polimorfizminin Büyüme Özellikleri ile İlişkisi

Araştırma Makalesi

Makale Tarihçesi:
Geliş tarihi: 31.03.2024
Kabul tarihi:08.07.2024
Online Yayınlanma: 10.12.2024

ÖZ

Bu çalışmada Osmaniye ilinde halk elinde ıslah projesi kapsamında yetiştiriciliği yapılan İvesi ve Akkaraman koyunlarında Kalpastatin geni (*CAST*) polimorfizminin belirlenmesi amaçlanmıştır. Çalışma 5 farklı işletmede yetiştirilen 20 baş İvesi koyundan ve yine 5 farklı işletmede yetiştirilen 19 baş Akkaraman koyundan alınan

Anahtar Kelimeler:
CAST geni
İvesi koyunu
Akkaraman koyunu
Polimorfizm
Genotip frekansı

toplam 39 kan örneği üzerinde yürütülmüştür. PCR-RFLP yönteminin kullanıldığı çalışmada, CAST geni için İvesi ve Akkaraman koyun ırklarına ait genomik DNA örneklerinden elde edilen 622 bç uzunluğundaki PCR amplikonlarının *Msp*I restriksiyon enzimi ile kesimi sonucunda; homozigot MM alleli için 336 ve 286 bç uzunluğunda iki adet bant, homozigot NN alleli için 622 bç uzunluğunda tek bir bant ve heterozigot MN allelleri için ise 622, 336 ve 286 bç uzunluğunda üç adet bant gözlenmiştir. Akkaraman ırkında M ve N allellerinin frekansı sırasıyla 0,82 ve 0,18 olarak hesaplanırken, İvesi ırkında M ve N frekansları sırasıyla 0,75 ve 0,25 olarak hesaplanmıştır. MM, MN ve NN genotiplerinin frekansları ise sırasıyla 0.60, 0,30 ve 0,10 olarak bulunmuştur. İvesi ırkının aksine Akkaraman koyunlarında sadece MM ve MN genotipleri gözlenmiş ve frekansları sırasıyla 0,63 ve 0,37 olarak hesaplanmıştır. Akkaraman popülasyonunda Hardy-Weinberg dengesinde sapma önemsiz bulunurken, İvesi koyununda önemli bulunmuştur. İlişki analizi İvesi ırklarında *CAST* geni polimorfizmi ile 90. gün ağırlığı arasında önemli bir ilişki olduğunu (P<0,05) ortaya koyarken, Akkaraman ıkında bu ilişki bulunamamıştır (P<0,05).

To Cite: Dokgöz M., Özcan BD. Relationship of Calpastatin Gene Polymorphism with Growth Traits in Some Locally Reared Awassi and Akkaraman Sheep Breeds. Osmaniye Korkut Ata Üniversitesi Fen Bilimleri Enstitüsü Dergisi 2024; 7(5): 2364-2373.

1. Introduction

Skeletal muscle mass is a significant economic trait, and the development and growth of muscles are crucial factors in providing sufficient meat for human consumption. Therefore, identifying the candidate genes that regulate skeletal muscle development is essential for comprehending the molecular genetic regulation of muscle growth (Mohammadabadi et al., 2021).

Palmer et al. (2000) and Khederzadeh (2011) reported that the Calpastatin gene (*CAST*) is located on the 5th chromosome in sheep. Calpastatin, consisting of five main domains, has a molecular weight of approximately 76 kDa (Suleman et al., 2012). Among these domains, the N-terminal leader (L) is thought to play no role in suppressing calpains (Emori et al., 1987), but it may contribute to determining the region where it settles within the cell (Averna et al., 2001). The other four domains are highly homologous and tend to suppress calpains (Emori et al., 1987; Cong et al., 1998). Within these domains, three regions, namely A, B, and C, have been identified. Regions A and C tightly bind to calpains with Ca^{+2} ions, but there is no calpain suppression activity in any of these regions (Todd et al., 2003).

The significance of animal protein in human nutrition is undeniable. Sheep have contributed to humanity for thousands of years with their yield characteristics such as meat, milk, and fleece, and continue to do so. Understanding the importance of this, people have tried to breed animals and plants involved in agricultural activities and have carried out studies to increase the quantity and quality of the products obtained from them. The *CAST* gene is one of the candidate genes affecting muscle growth and is involved in regulating the firmness of meat after slaughter (Zhou et al., 2007). While this suggests that the *CAST* gene is more involved in meat quality, there is also a link between muscle growth rate and Calpastatin activity. Muscle growth is due to a reduction in protein degradation as a result of Calpastatin activity. Thus, it appears that the *CAST* gene has a positive effect on meat yield in addition to meat quality. Amanda et al. (2004) also stated that the *CAST* gene should be of particular interest in studies to improve meat quality and body weight gain in livestock. Calpastatins inhibit endogenous biosynthesis and degradation of calpains (Khederzadeh, 2011). The *CAST* gene can be classified as a candidate gene

due to the presence of common genomic regions in livestock (sheep, goats, pigs, cattle, etc.) and also in rodent mouse (Hitomi et al., 2000). Palmer et al. (1998) determined two different (M and N) alleles of the *CAST* gene in Dorset sheep by PCR-RFLP method. These two alleles of the *CAST* gene were also determined in subsequent studies in different breeds (Shahroudi et al., 2006; Mohammadi et al., 2008; Szkudlarek-Kowalczyk et al., 2011; Khan et al. 2012).

This study was carried out to determine the *CAST* gene polymorphism by PCR-RFLP method at the DNA level in Awassi and Akkaraman sheep raised in Osmaniye province. Furthermore, the aim was to assess the association between the genotypes determined for the CAST gene in Akkaraman and Awassi sheep breeds and sheep growth characteristics.

2. Material and Method

2.1. Animals and sample collection

The animal material of the study consisted of a total of 39 animals, which consisted of 20 Awassi and 19 Akkaraman from a total of 10 sheep farms under the National Sheep Breeding Project in the Hand of the Public reared in different locations in Osmaniye province of Türkiye. Blood samples were collected from the *Vena jugularis* of the animal material using a vacutainer tube containing K3EDTA and stored at –20 °C until analyses. The genomic DNA was extracted using GeneJET Whole Blood Genomic DNA Purification Mini Kit (Thermo Fisher Scientific) following the procedure given in the user manual. DNA quality and quantity were assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA).

Table 1. Primer sequences of the *CAST* gene locus (Oligomer, Ankara, Türkiye)

Primer	Sequences (5' → 3')	G+C Content (%)	Tm (°C)	Reference
CAST-F	CCTTGTCATCAGACTTCACC	50	60	Khederzadeh (2011)
CAST-R	ACTGAGCTTTTAAAGCCTCT	40		

2.2. PCR amplification

A 622 bp fragment of the *CAST* gene was amplified by polymerase chain reaction (PCR) using the primers listed in Table 1. The PCR reaction was carried out in a 50 µL volume using 1 µL (200 ng) of DNA, 5 µL of PCR reaction buffer, 1 µL for each primer (forward and reverse, 20 pmol each), 4 µL of dNTP mix (10 mM), 3 µL of $MgCl_2$ (25 mM), 0.5 µL of Taq DNA polymerase (5 u/µL, Thermo Fisher Scientific) and 34.5 µL of distilled water. The thermal cycling conditions were as follows: initial denaturation at 95°C for 5 min; 30 cycles of 95°C for 1 min, 60 °C annealing for 1 min, and 72 °C extension for 2 min; final extension at 72 °C for 10 min. The PCR products were visualized by electrophoresis on a 0.8% agarose gel stained with ethidium bromide.

2.3. RFLP genotyping

The 622 bp PCR amplicons were genotyped by restriction fragment length polymorphism (RFLP) method using the *Msp*I restriction enzyme (Thermo Fisher Scientific, USA, Cat. No: ER0541). The PCR products were digested using *Msp*I restriction enzymes at 37°C, for 60 minutes. The reaction mixture consisted of 5 μL of PCR products, 3.5 μL of distilled water, 1 μL of 10X buffer, and 0.5 μL of the restriction enzymes. The digested PCR products were separated by electrophoresis on a 1% agarose gel stained with ethidium bromide and visualized under UV light (Vilber Lourmat, Germany).

2.4. Calculation of allele and genotype frequencies

After determining the genotype of each individual and documenting them in the gel images, gene and genotype frequencies were calculated using the direct counting method for Calpastatin genetic variants. Gene and genotype frequencies were calculated separately for two herds and also for the whole population.

2.5. Statistical analysis

The $\chi 2$ (chi-square) test was used to determine whether the herds and the population were in Hardy-Weinberg equilibrium. Yates continuity correction was applied to genotypes with sample sizes of 5 or less. One-way analysis of variance (ANOVA) was used to determine the relationships between gene polymorphisms and growth traits. Tukey test was used for genotypic association analysis to determine the significance of differences in growth traits following ANOVA. Descriptive statistics of the growth efficiency of the studied individuals are shown with mean and standard error. All statistical analyses were performed using the Minitab 19.1 software package, and a significance level of $P<0.05$ was adopted.

3. Results and Discussion

CAST gene with a length of 622 bp was amplified by PCR from the genomic DNA of Akkaraman and Awassi sheep. After digestion of the amplicons with *Msp*I restriction enzyme, two bands of 336 and 286 bp in length for the homozygous MM allele, a single band of 622 bp in length for the homozygous NN allele, and three bands of 622, 336, and 286 bp in length for heterozygous MN alleles were observed in agarose gel (Figure 1).

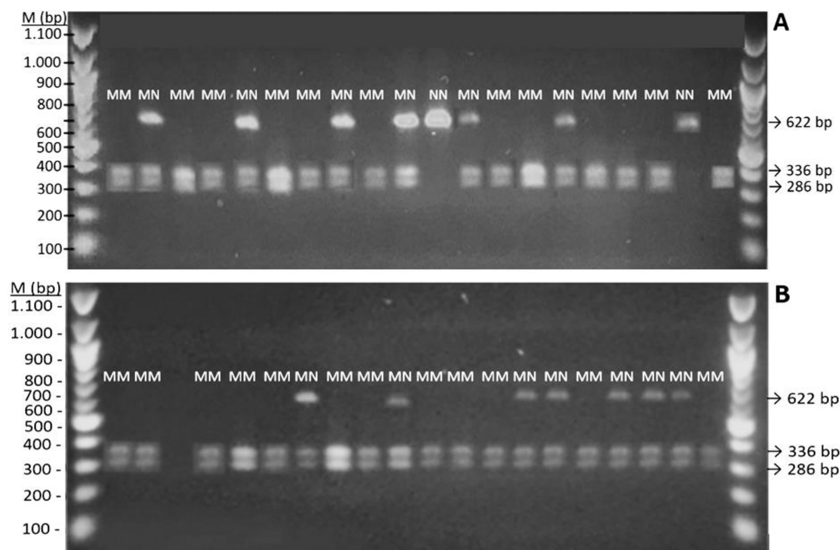

Figure 1. Genotypes of the *CAST* gene of Awassi (A) and Akkaraman (B) sheep bred on an agarose gel. MM (336 and 286 bp), MN (622, 336 and 286 bp), NN (622 bp) (M: 100 bp DNA ladder, Thermo Fisher Scientific, SM0241)

The obtained results revealed that both Awassi and Akkaraman sheep breeds reared by local sheepherd in Osmaniye are polymorphic in terms of the *CAST* gene. It was observed that M and N alleles of the *CAST* gene were present with varying frequencies in the Awassi and Akkaraman sheep breeds. While the frequencies of M and N alleles in the Akkaraman breed were calculated as 0.82 and 0.18, respectively, the frequencies in the Awassi breed were calculated as 0.75 and 0.25, respectively (Table 2). Homozygous MM and heterozygous MN genotypes were observed in both breeds, while homozygous NN allele was observed only in the Awassi breed.

Table 2. Genotype and allele frequencies of the *CAST* gene in Awassi and Akkaraman sheep breed

Breed	N*	Genotype frequency			Allele frequency	
		MM	MN	NN	M	N
Awassi	20	0.60	0.30	0.10	0.75	0.25
Akkaraman	19	0.63	0.,37	0	0.82	0.18
Total	39					

*Number of animals

While deviation from Hardy-Weinberg equilibrium was found to be insignificant in the Akkaraman population, significant deviation from Hardy-Weinberg equilibrium was observed in the Awassi sheep and the total population when the chi-square test was applied. Association analysis results revealed that there was a significant relationship between *CAST* gene polymorphism and 90th-day weight in Awassi breeds ($P<0.05$). The 90th-day weight averages of the genotypes were MM (24.45±0.17), MN (23.70±0.24), and NN (24.72±0.62) (Table 3). It was determined that the individuals with the NN genotype had a higher mean 90th-day weight maintenance than the other individuals. However, there was no relationship between *CAST* gene polymorphism and growth traits in Akkaraman breeds ($P>0.05$) (Table 3).

Table 3. Association analysis between *CAST* gene and growth traits in Awassi and Akkaraman breeds

Breed	Description	Genotype			P value
		MM	MN	NN	
Awassi	Birth weight (kg)	3.41±0.24	3.43±0.23	3.42±0.31	0.985
	60th Day weight (kg)	16.41±0.60	16.13±0.29	16.98±0.32	0.152
	90th Day weight (kg)	24.45±0.17[a]	23.70±0.24[b]	24.72±0.62[a]	**0.006***
Akkaraman	Birth weight (kg)	4.12±0.22	4.06±0.30	-	0.602
	60th Day weight (kg)	17.20±0.72	16.74±0.50	-	0.160
	90th Day weight (kg)	24.42±1.28	23.78±0.71	-	0.244

In this study in which *CAST* gene polymorphism was determined in Awassi and Akkaraman sheep breeds raised in Osmaniye within the scope of the National Sheep Breeding Project in the Hand of the Public, M, and N allele frequencies in Akkaraman sheep were calculated as 0.82 and 0.18, respectively, while the frequencies in Awassi sheep were calculated as 0.75 and 0.25, respectively. In previous studies on another population of Akkaram sheep, Kırıkçı (2021) reported M and N allele frequencies as 0.68 and 0.32, respectively, while similar studies reported M and N allele frequencies as 0.69 and 0.31 (Balcıoğlu et al., 2014), 0.90 and 0.10 (Bayram et al., 2019). In a study on the Awassi breed, M and N allele frequencies were reported as 0.59 and 0.41, respectively (Balcıoğlu et al., 2014). These data show that the M and N alleles of the *CAST* gene in Akkaraman and Awassi sheep in Turkey generally show similar frequencies and the M allele frequency is higher than the N allele frequency. This similarity is not limited to Akkaraman and Awassi sheep breeds. In other domestic sheep breeds in which *CAST* gene polymorphism was investigated, M and N allele frequencies were reported as 0.92 and 0.08 in Kangal breed, 0.67 and 0.33 in Güney Karaman breed, 0.87 and 0.13 in Morkaraman breed, 0.86 and 0.14 in Karayaka breed, 0.89 and 0.11 in Karakaş breed (Balcıoğlu et al., 2014). In another study, the frequencies of M and N alle were reported as 0.70-0.30, 0.963-0.037, 0.909-0.091, 0.895-0.105, 0.733-0.267 and 0.75-0.25 in Kıvırcık, İmroz, Karayaka, Hemşin, Karakul and Morkaraman breed sheep, respectively (Avanus, 2015). Kaplan and Atalay (2017) reported M and N allele frequencies as 0.90 and 0.10 in Kıvırcık crossbred sheep. In studies conducted in other countries, the frequencies of M allele in local breeds were generally reported to be high (Mohammadi et al., 2008; Gabor et al., 2009; Szkudlarek-Kowalczyk et al., 2011; Khederzadeh, 2011; Khan et al., 2012; Suleman et al., 2012). However, in the *CAST*/*Msp*I polymorphism study conducted in the Chios breed, the M allele frequency was found to be as low as 0.35 (Yılmaz et al., 2014), which is not a common situation. In the Awassi breed, frequencies of MM, MN, and NN genotypes for *CAST*/*Msp*I were found to be 0.60, 0.30, and 0.10, respectively. On the other hand, only MM and MN genotypes were observed in Akkaraman sheep and their frequencies were 0.63 and 0.37, respectively. While the frequency of the NN genotype was found to be very low in Awassi breed sheep, the NN genotype was not observed in Akkaraman breed sheep. Similar results were reported for Gökçeada (Yılmaz et al., 2014), Ost Friz (Gabor et al., 2009), Kangal (Balcıoğlu et al., 2014), Balkhi (Khan et al., 2012), Thalli (Suleman et al., 2012), Berichon du Cher and Ile de France (Szkudlarek-Kowalczyk et al., 2011) sheep breeds. In another study conducted on the Akkaraman breed, the NN genotype frequency was reported as a meager rate of 0.02 (Kırıkçı,

2022). This frequency value is very similar to the NN frequency obtained for Akkaraman sheep in this study. On the other hand, low MN genotype frequencies were observed in both Awassi and Akkaraman sheep in our study. These results indicate that homozygosity in favor of M allele occurred in both populations. Because the animals used in this study were selected from elite and closed flocks, it is expected to show different frequencies from the results obtained in other studies. Because the absence of the NN genotype in the Akkaraman sheep breed indicates the closed natüre of the flock. On the other hand, the similarity with the results determined in different studies is the result of the fact that the studied gene is a conserved region between the breeds. The fact that the Awassi breed is not in Hardy-Weinberg equilibrium can be explained by homozygotization due to the closed herds. When the obtained genotype frequencies and Hardy-Weinberg equilibrium results are evaluated together, considering that the 90th-day live weight was also found to be significant, the *CAST* gene can be considered as a candidate gene associated with growth, especially for the Awassi breed and can be used in marker-assisted selection studies.

The results of the association analysis showed that there was a significant association between *CAST* gene polymorphism and 90th-day weight in Awassi breed animals ($P<0.05$). However, no association was found between *CAST* gene polymorphism and birth weight and 60th-day weight in Awassi breed sheep and birth, 60th-day, and 90th-day weight in Akkaraman breed sheep ($P>0.05$). It was determined that the individuals with the NN genotype in the Awassi breed had a higher mean 90th-day weight than the other individuals. However, Jawasreh et al. (2017) reported that individuals with the MN genotype recorded higher body weight gain than individuals with the MM genotype in a study conducted in Awassi breed sheep. Similarly, Chung and Davis (2012) conducted a study on the *CAST* gene in Polypay, Targhee, and crossbred ewes and found that *CAST* gene polymorphism was effective on body weight gain and birth weight. However, there are also reports in the literature that individuals with the NN genotype have lower body weight than other genotypes (Khan et al., 2012). Bayraktar and Shoshin (2022) reported that *CAST* gene polymorphism was associated with body weight, body length, cidago height, and chest depth in a study conducted in Awassi breed animals.

The *CAST* gene is known to be a candidate gene with a major effect on meat quality and tenderness. At the same time, the findings obtained from this study and other similar studies show that there is a relationship between *CAST*/*Msp*I polymorphism and growth traits. These studies suggest that the *CAST* gene can be taken into consideration in future genetic breeding studies and included in breeding programs.

Conclusion

In the study on the determination of *CAST* gene polymorphism by PCR-RFLP method in Awassi and Akkaraman sheep raised in Osmaniye province, it was determined that animals were polymorphic in terms of *CAST*/*Msp*I locus in both sheep breeds. While the frequency of individuals with MM genotype was high in both breeds, individuals with NN genotype were not observed in Akkaraman breed. This

study also revealed the CAST/MspI locus variation in both breeds. The findings of association analysis revealed that individuals with the NN genotype in the Awassi breed reached higher 90th-day live weight. These findings will contribute to genetic improvement by including the *CAST* gene in future breeding studies in terms of the aforementioned characters.

Acknowledgements

The authors acknowledge the Çukurova University Research Fund (FYL-2019-12464) for support to carry out this study.

Conflict of Interest Statement

The authors declare that there is no conflict of interest.

Summary of Researchers' Contribution Rate Declaration

The authors declare that they have made a similar contribution to the article.

Kaynakça:

Amanda W., Thompson VF., Goll DE. Interaction of calpastatin with calpain: a review. Biological Chemistry 2004; 385: 465-472.

Avanus K. Genetic variability of CAST gene in native sheep breeds of Turkey. Kafkas Üniversitesi Veteriner Fakültesi Dergisi 2015; 21(6): 789-794.

Averna M., Tullio RD., Passalacqua M., Salamini F., Ponteremoli S., Mellon E. Changes in intracellular calpstatin localization are mediated by reversible phosphorylation. Biochemical Journal 2001; 354: 25-30.

Balcıoğlu MS., Karslı T., Şahin E., Ulutaş E., Aksoy Y. Türkiye'de yetiştirilen bazı yerli koyun ırklarında kalpastatin (CAST) geni polimorfizminin PCR-RFLP yöntemiyle belirlenmesi. Tarım Bilimleri Dergisi 2014; 20: 427-433.

Bayraktar M., Shoshin O. Association between *CAST* and *MSTN* gene polymorphisms with growth traits in Awassi sheep. Kuwait Journal of Science 2022; 49(2): 1-5.

Bayram D., Akyüz B., Arslan K., Özdemir F., Aksel EG., Çınar MU. *DGAT1*, *CAST* and *IGF-I* gene polymorphisms in Akkaraman lambs and their effects on live weights up to weaning age. Kafkas Üniversitesi Veteriner Fakültesi Dergisi 2019; 25 (1): 9-15.

Chung H., Davis M. PCR-RFLP of the ovine calpastatin gene and its association with growth. Asian Journal of Animal and Veterinary Advances 2012; 7: 641-652.

Cong M., Thompson VF., Goll DE., Antin PB. The bovine calpastatin gene promoter and a new N-terminal region of the protein are targets for campdependent protein kinase activity. Journal of Biological Chemistry 1998; 273: 660-666.

Emori Y., Kawasaki H., Imajoh S., Imahori K., Suzuki K. Endogenous inhibitor for calcium-dependent cysteine protease contains four internal repeats that could be responsible for its multiple reactive sites. PNAS of the United States of America 1987; 84: 3590-3594.

Gábor M., Trakovická A., Miluchová M. Analysis of polymorphism of *CAST* gene and *CLPG* gene in sheep by PCR-RFLP method. Scientific Papers: Animal Science and Biotechnologies 2009; 42(2): 470-476.

Hitomi K., Murase M., Kawamura T., Maki M. Constant expression of mouse calpastatin isoforms during differentiation in myoblast cell line, C2C12. Cytotechnology 2000; 33: 63-70.

Jawasreh KI., Jadallah R., Al-Amareen AH., Abdullah AY., Al-Qaisi A., Alrawashdeh IM., Al-Zghoul MBF., Ahamed MKA., Obeidat B. Association between *Msp*I calpastatin gene polymorphisms, growth performance, and meat characteristics of Awassi sheep. Indian Journal of Animal Sciences 2017; 87(5): 635-639.

Kaplan S., Atalay S. Determination of calpastatin gene polymorphism in Kıvırcık crossbred ewes by PCR-RFLP method. Selcuk Journal of Agriculture and Food Sciences 2017; 31(3): 147-150.

Khan SUH., Riaz MN., Ghaffar A., Khan MFU. Calpastatin (*CAST*) gene polymorphism and its association with average daily weight gain in Balkhi and Kajli sheep and Beetal goat breeds. Pakistan Journal of Medical Sciences 2012; 44: 377-382.

Khederzadeh S. Polymorphism of calpastatin gene in crossbreed Dalagh sheep using PCR-RFLP. African Journal of Biotechnology 2011; 10: 10839-10841.

Kırıkçı K., Mehmet CAM., Mercan L. Investigation of the *CAST* gene polymorphism in Karayaka sheep. Manas Journal of Agriculture Veterinary and Life Sciences 2021; 11(1): 89-93.

Mohammadabadi M., Bordbar F., Jensen J., Du M., Guo W., Key G. Genes regulating skeletal muscle development and growth in farm animals. Animals 2021; 11(3): 1-25.

Mohammadi M., Nasiri MTB., Alami-Saeid KH., Fayazi J., Mamoee M., Sadr AS. Polymorphism of calpastatin gene in Arabic sheep using PCR-RFLP. African Journal of Biotechnology 2008; 7(15): 2682-2684.

Palmer BR., Roberts N., Hickford JGH., Bickerstaffe R. Rapid communication: PCR-RFLP for *Msp*I and *Nco*I in the ovine calpastatin gene. Journal of Animal Science 1998; 76: 1499-1500.

Palmer BR., Su HY., Roberts N., Hickford JGH., Bickerstaffe R. Single nucleotide polymorphisms in an intron of the ovine calpastatin gene. Animal Biotechnology 2000; 11: 63-67.

Shahroudi FE., Nassiry MR., Valizadh R., Moussavi AH., Pour MT., Ghiasi H. Genetic polymorphism at *MTNR1A*, *CAST* and *CAPN* loci in Iranian Karakul sheep. Iranian Journal Biotechnology 2006; 4: 117-122.

Suleman M., Khan SU., Riaz MN., Yousaf M., Shah A., Ishaq R., Ghafoor A. Calpastatin (*CAST*) gene polymorphism in Kajli, Lohi and Thalli sheep breeds. African Journal of Biotechnology 2012; 11(47): 10655-10660.

Szkudlarek-Kowalczyk M., Wiśniewsk E., Mroczkowsk S. Polymorphisms of calpastatin gene in sheep. Journal of Central European Agriculture 2011; 12: 425-432.

Todd B., Moore D., Deivanayagam CCS., Lin G., Chattopadhyay D., Maki M., Wang KKW., Narayana SVL. A structural model for the inhibition of calpain by calpastatin: Crystal structures of the native domain VI of calpain and its complexes with calpastatin peptide and a small molecule inhibitor. Journal of Molecular Biology 2003; 328(1): 131-146.

Yılmaz O., Sezenler T., Ata N., Yaman Y., Cemal İ., Karaca O. Polymorphism of the ovine calpastatin gene in some Turkish sheep breeds. Turkish Journal of Veterinary and Animal Sciences 2014; 38: 354-357.

Zhou H., Hicford JGH., Gong H. Polymorphism of the ovine calpastatin gene. Molecular and Cellular Probes 2007; 21: 242-244.