Research Article

www.dergipark.org.tr/en/pub/mediterranean

MEDITERRANEAN AGRICULTURAL SCIENCES
(2025) 38(2): 103-108
DOI: 10.29136/mediterranean.1712441

Determination of *CAST*/*Msp*I gene polymorphism in selected sheep breeds reared in Türkiye*

Goknur BAYRAK, Yasemin GEDIK

Eskisehir Osmangazi University, Faculty of Agriculture, Department of Animal Science, Eskişehir, 26480, Türkiye

Corresponding author: Y. Gedik, e-mail: ygedik@ogu.edu.tr
Author(s) e-mail: goknurcap@gmail.com

ARTICLE INFO

Received: June 3, 2025
Received in revised form: June 30, 2025
Accepted: July 1, 2025

***Keywords*:**

Calpastatin gene
*Msp*I
Polymorphism
Sheep

ABSTRACT

Sheep breeding plays a crucial role in meeting the increasing demand for high-quality meat, milk, and wool. Traditional selection methods based on phenotypic traits have led to genetic improvement; however, progress remains slow for traits with low to moderate heritability. Marker-assisted selection (MAS) has emerged as a promising complementary approach, enabling faster genetic gains. Among candidate genes studied for MAS, the calpastatin (*CAST*) gene is notable for its association with meat yield and quality. This study aimed to identify *CAST/Msp*I gene polymorphisms in four sheep breeds reared in Türkiye (Central Anatolian Merino-CAM, Pırlak-PRL, Romanov-RMV, and Suffolk-SFK) and assess their potential use in MAS. A total of 176 individuals were genotyped using the PCR-RFLP method. All breeds in this study were found to be polymorphic for the *CAST* gene. The frequency of the M allele ranged from 0.67 in CAM to 0.76 in SFK. Genotype frequencies for MM ranged from 0.40 (CAM) to 0.71 (SFK), for MN from 0.10 (RMV and SFK) to 0.54 (CAM), and for NN from 0.06 (CAM) to 0.20 (RMV). Significant deviations from Hardy-Weinberg equilibrium were observed in CAM, RMV, and SFK populations, but not in PRL. Observed heterozygosity ranged from 0.10 to 0.54 and expected heterozygosity from 0.37 to 0.44. The presence of all three genotypes and substantial genetic variation suggests that the *CAST* gene may be a valuable marker in MAS for these breeds. However, further association studies are required to confirm the relationship between *CAST* genotypes and economically significant traits related to meat production.

1. Introduction

Traditional selection methods, which have been employed for many years to increase yields in animal production, are often slow, laborious, labor-intensive, and costly. Most economically important traits in livestock are quantitative in nature, characterized by low heritability and influenced by a multitude of genes as well as environmental factors. Genetic progress tends to be slower for phenotypic traits such as meat or milk yield, which typically have low heritability and can only be measured in the later stages of an animal's life. However, the early identification of candidate genes associated with various performance traits, and their subsequent application in marker-assisted selection (MAS) programs, can significantly accelerate genetic improvement (Karslı et al. 2017; Demir et al. 2022). Identifying candidate genes suitable for MAS is a complex process that requires establishing associations between genotypic and phenotypic data (Javanmard et al. 2010; Kania et al. 2019). Calpastatin (*CAST*) gene is one of the most studied genes reported to be associated with meat yield and quality in different livestock species (Javanmard et al. 2010; Ropka-Molik et al. 2014; Kania et al. 2019).

In sheep, approximately one-quarter of the total body weight consists of skeletal muscle. Skeletal muscle growth in animals is primarily determined by three factors: the number and size of muscle cells, the rate of muscle protein synthesis, and the rate of protein degradation (Ibrahim et al. 2015). Various physiological and genetic factors influencing muscle growth act upon one or more of these fundamental mechanisms. However, in domestic animals, variations in the rate of muscle growth are more commonly attributed to differences in protein degradation rather than changes in protein synthesis (Goll et al. 1998).

Muscle protein degradation, or proteolysis, is mediated by several well-characterized proteolytic enzyme systems, among which the calpain-calpastatin system (CCS) is particularly prominent. This system comprises calpains, a family of calcium-dependent cysteine proteases, with at least fifteen isoforms identified and their endogenous inhibitor, calpastatin. The role of the CCS has been well established in both normal and postnatal skeletal muscle development, as well as in muscle wasting conditions. In general, increased skeletal muscle growth is associated with reduced rates of protein degradation and decreased calpain activity, primarily resulting from elevated calpastatin activity (Goll et al. 1998). Importantly, the calpain-to-calpastatin ratio is considered to be a key determinant of muscle accretion in animals (Kania et al. 2019; Valencia et al. 2022). In contrast, during the post-mortem conversion of muscle to meat, reduced calpastatin activity permits enhanced proteolysis, which significantly contributes to meat tenderization (Kawasaki and

*Ethical approval and permission for this study was obtained from Eskisehir Osmangazi University Animal Experiments Local Ethics Committee (Date: 09/10/2020; Decision No: 809).

Kawashima 1996; Goll et al. 1998; Ciobanu et al. 2004; Jawasreh et al. 2019).

The *CAST* gene, which plays a critical role in post-mortem meat tenderization and skeletal muscle development, is located on chromosome 5 in sheep and comprises 29 exons spanning a total of 89576 base pairs (NCBI 2021). Polymorphisms in the *CAST* gene have been reported to be associated with several economically important traits, including birth weight, growth performance, growth rate, muscle development, and carcass yield. In addition to these production traits, variations in *CAST* have also been linked to meat quality characteristics such as pH, meat color, cooking loss, and tenderness (Palmer et al. 1997; Chung and Devis 2012; Knight et al. 2012; Ramadevi et al. 2020). In this context, the aim of the present study was to identify *CAST/Msp*I gene polymorphisms in four sheep breeds in Türkiye (CAM, PRL, RMV, and SFK) and to evaluate their possibilities for use in MAS studies.

2. Materials and Methods

Ethical approval and permission for this study was obtained from Eskisehir Osmangazi University Animal Experiments Local Ethics Committee (Date: 09/10/2020; Decision No: 809).

To carry out CAST gene polymorphism in sheep breeds, blood samples were taken from the jugular vein of animals into sterile vacuum tubes containing EDTA. Information about the blood samples used in the study (breed, number of samples and where collected) is shown in Table 1. Genomic DNA from whole blood was extracted using a commercial DNA isolation kit (BLIRT DNA isolation kit, EM13-250) according to the manufacturer's instructions. DNA quality and quantity were determined using gel electrophoresis (1%) and ND 1000 NanoDrop spectrophotometer (A260/A280 nm).

Table 1. Sampling regions and sample sizes

District	Breed (Abbreviation)	Sample size (n)
Çifteler	Central Anatolian Merino (CAM)	32
	Pırlak (PRL)	8
	Romanov (RMV)	10
	Suffolk (SFK)	21
Sivrihisar	Central Anatolian Merino (CAM)	64
Mahmudiye	Central Anatolian Merino (CAM)	41
Total		176

Polymorphisms in the *CAST* gene were determined in the studied sheep breeds using the PCR-RFLP method. After the DNA isolation step, the primers reported by Palmer et al. 1998 were utilized for the amplification of the *CAST* gene by PCR. To amplify the 622 bp region of the cast gene, 25 µl of reaction mixture was prepared by adding 100 ng DNA, 10 X PCR buffer, 0.6 mM of each dNTP, 1.5 mM $MgCl_2$, 1 pM of each primer and 1 U of Taq polymerase. The PCR amplification was performed using 35 cycles of 95˚C for 1min, 62˚C for 1min and 72˚C for 2 min, followed by 72˚C for 10 min. The 622 bp products were digested with *Msp*I restriction enzyme. The bands were visualized using ultraviolet transillumination and the size of the amplified fragments was compared to a Solis Biodyne 100 bp DNA ladder (Cat 07-11-00050).

Based on the results of electrophoresis, the presence of the relevant genotypes was identified. The Popgene software package was used to calculate allele and genotype frequencies, observed (Ho) and expected heterozygosity (He), and Hardy-Weinberg equilibrium for the CAST gene (Yeh et al. 1997). The chi-squared test ($\chi 2$) was also used to test whether or not the populations were in Hardy-Weinberg equilibrium.

3. Results and Discussion

PCR amplification successfully produced 622 bp *CAST* gene fragments, and a 100 bp ladder was used for comparison of the amplification length. The PCR products were then digested with the restriction enzyme *Msp*I and separated on 2% agarose gel electrophoresis. As a result of PCR-RFLP, the presence of polymorphism in *CAST* gene presented two alleles M and N. Enzyme *Msp*I produced two fragments of 336 bp and 286 bp for allele M, whereas the PCR product remained uncut for allele N. The PCR-RFLP profile of M allele homozygous animals (MM) showed two bands of 336 and 286 bp. All three genotypes were observed, and the *CAST* gene locus was found to be polymorphic in all populations studied. The heterozygous genotype (MN) showed three bands of 622, 336 and 286 bp and homozygous for the N allele (NN) showed only one band of 622 bp, as shown in Figure 1.

The gene frequencies of the *CAST* locus were estimated using both the Popgene V.1.31 (Yeh et al.1997) software package and counting the number of genes. The chi-squared test (χ^2) was used to test whether or not the populations were in Hardy-Weinberg

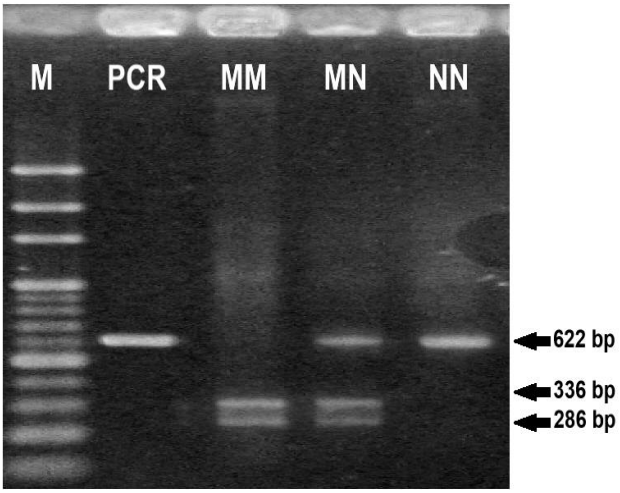

Figure 1. Agarose gel image of *CAST* genotypes detected using the PCR-RFLP.

equilibrium. The frequencies of genotypes and alleles of the *CAST* locus are shown in Table 2.

The estimated genetic diversity parameters for the *CAST* gene in the studied sheep breeds are presented in Table 3.

In all breeds, except the Central Anatolian Merino, the homozygous MM genotype had the highest frequency, and M was the most common allele. The allele frequencies reported from previous studies on different sheep breeds are summarized in Table 4.

The calculated allele frequencies (M and N) in the Pırlak, Romanov and Suffolk sheep breeds were consistent with studies by Shahroudi et al. (2006) in Karakul breed, Szkudlarek-Kowalczyk (2011) in Polish merino, Gharahveysi et al. (2012) in Zel breed, Tohidi (2013) in Sanjabi sheep, Sunilkumar et al. (2014) in Bandur sheep, Avanus et al. (2015) in Karakul sheep, Bozhilova-Sakova et al. (2020) in Northeast Bulgarian Merino breed and Bayraktar and Shoshin (2022) in Awassi sheep. The

Table 2. Genotype and allele frequencies of CAST gene

Breed	N	Genotype Frequencies			Allele Frequencies	
		MM	MN	NN	M	N
Central Anatolian Merino	137	0.40 (55)	0.54 (74)	0.06 (8)	0.67	0.33
Pırlak	8	0.63 (5)	0.25 (2)	0.12 (1)	0.75	0.25
Romanov	10	0.70 (7)	0.10 (1)	0.20 (2)	0.75	0.25
Suffolk	21	0.71 (15)	0.10 (2)	0.19 (4)	0.76	0.24

Table 3. Genetic diversity parameters in the studied breeds

Breed	n	Ho	He	Ne	χ^2
Central Anatolian Merino	137	0.54	0.44	1.78	6.86
Pırlak	8	0.25	0.40	1.60	0.88
Romanov	10	0.10	0.39	1.60	5.37
Suffolk	21	0.09	0.37	1.56	12.64

n: sample size, Ho: Observed heterozygosity; He: Expected heterozygosity, Ne: Number of effective alleles. $\chi^2_{1,0.05}$: 3.841 Hardy-Weinberg equilibrium (P<0.05).

Table 4. The allele frequencies in different sheep breeds

Breed	Allele Frequencies		Reference
	M	N	
Karagül	0.79	0.21	Shahroudi et al. (2006)
Tsigai x Lacaune	0.90	0.10	Gabor et al. (2009)
Tsigai	0.91	0.09	
Polonya Merinos	0.76	0.24	Szkudlarek-Kowalczyk et al. (2011)
Berrichon du Cher	0.92	0.08	
Ile de France	0.95	0.05	
Atabi	0.64	0.36	Nanekarani et al. (2011)
Zel	0.75	0.25	Gharahveysi et al. (2012)
Balkhi	0.88	0.12	Khan et al. (2012)
Kajli	0.86	0.14	
Lohi	0.87	0.13	Suleman et al. (2012)
Kajli	0.81	0.19	
Thalli	0.90	0.10	
Sanjabi	0.72	0.28	Tohidi (2013)
Ghezel	0.69	0.31	
Afshari	0.63	0.37	
Makui	0.88	0.12	
Bandur	0.72	0.28	Sunilkumar et al. (2014)
Lori	0.63	0.37	Asadi et al. (2014)
Güney Karaman	0.67	0.33	Balcıoğlu et al. (2014)
Akkaraman	0.69	0.31	
Awassi	0.59	0.41	
Kangal	0.92	0.08	
Karayaka	0.89	0.11	
Morkaraman	0.87	0.13	
Gökçeada	0.99	0.01	Yılmaz et al. (2014a)
Kıvırcık	0.85	0.15	
Karacabey Merino	0.80	0.20	
Kıvırcık	0.84	0.16	Yılmaz et al. (2014b)
Karagül	0.73	0.27	Avanus et al. (2015)
Shumen	0.92	0.08	Georgieva et al. (2015)
Northeast Bulgarian Merino	0.73	0.27	Bozhilova-Sakova et al. (2020)
Awassi	0.78	0.22	Bayraktar and Shoshin (2022)

allele frequencies calculated in Central Anatolian Merino sheep breed were similar to the results of Nanekarani et al. (2011) in Atabi sheep, Tohidi (2013) in Ghezel and Afshari sheep, Asadi et al. (2014) in Lori sheep and Balcıoğlu et al. (2014) in Güney Karaman, Akkaraman and Awassi sheep breeds.

However, this study is not in agreement with the following studies:- Gabor et al. (2009) in Tsigai x Lacaune and Tsigai sheep, Szkudlarek-Kowalczyk et al. (2011) in Berrichon du Cher and Ile de France sheep, Khan et al. (2012) in Balkhi and Kajli sheep, Suleman et al. (2012) in Lohi, Kajli and Thalli sheep, Tohidi (2013) in Makui sheep, Balcıoğlu et al. (2014) in Kangal, Karayaka and Morkaraman sheep, Yılmaz et al. (2014a, 2014b) in Gökçeada, Kıvırcık, Karacabey merino sheep and Georgieva et al. (2015) in Shumen sheep. The frequency of the M allele in these studies is considerably higher than in the results of this study.

There was no statistical significance between the observed and expected frequency differences for the *CAST* gene in the Pırlak population. In terms of the *CAST* polymorphism, the Pırlak population was found to be in Hardy-Weinberg genetic equilibrium (Table 3, $P<0.05$). However, the higher $\chi 2$ values calculated for the Central Anatolian Merino, Suffolk, and Romanov populations indicated that the $\chi 2$ values for these three breeds were significant (Table 3, $P<0.05$). The chi-square test showed that these sheep populations were not in Hardy-Weinberg equilibrium for the *CAST* locus. This unexpected result could be due either to a sampling error or to the fact that a few rams were used for breeding.

Polymorphisms in the *CAST* gene in sheep influence various growth traits and carcass traits. Most of the studies reported a positive effect of the MN genotype on the weight of the animals measured at different ages. In the same way, in different breeds, the MN genotype was reported to have a 15.4% higher birth weight than the NN genotype (Ramadevi et al. 2020; Valencia et al. 2022) and for weaning weight, MN was 1.04 kg higher than MM (Gorlov et al. 2016). It has also been reported that daily weight gain from birth to weaning is greater in MM than in MN and NN (Nassiry et al. 2006; Yilmaz et al. 2014b, Gorlov et al. 2016 and Jawasreh et al. 2017). However, Afanasyeva et al. (2019) reported that higher growth of MM is limited up to weaning in the West Siberian mutton sheep breeds. In most studies, the M allele was superior for all growth-related traits, with few exceptions, for example, in the Prydniprovska meat sheep and Altai Mountain breed, animal weight was in favour of the N allele rather than the M allele (Pomitun et al. 2019; Selionova et al. 2020). In addition, Jawasreh and Ismail (2019) reported that MN lambs of the Awassi breed may be healthier due to a higher neutrophils and neutrophil to lymphocyte (N/L) ratio, high triiodothyronine (T3) and cortisol in their blood hematology and serum studies.

In addition to growth traits, *CAST* variants influence carcass and meat quality in sheep. The pre-slaughter live weight of MN genotyped sheep from Volgograd was 3.7 kg more than that of MM genotyped sheep (Kolosov et al. 2021). Palmer et al. (1999) found that the ac genotype had a 15-18% higher age-corrected carcass weight and MN had a higher chilled carcass weight. Furthermore, the aa genotype had the highest percentage of muscle and lowest percentage of fat in the hindquarters, while the ac genotype had the highest intramuscular fat in the ram loin of the two synthetic breeds (Kania et al. 2019). Ibrahim et al. (2015) also reported that the lean and fat percentages of sheep meat were also influenced by the *CAST* gene. MM and MN genotypes tended to have the highest longissimus muscle width, and MN had heavier longissimus muscle than MM (Jawasreh et al. 2017; 2019). MM and MN had higher backfat thickness and skin plus backfat thickness values of the loin eye muscle in Kıvırcık sheep than in NN genotypes (Yilmaz et al. 2014b). In a study by Selionova et al. (2020), N was found to be superior (NN genotype) in carcass mass, carcass performance and meat percentage in the Altai Mountain breed.

Kumar et al. (2018) found that the Warner-Bratzler shear force score of the NN genotype was significantly ($P<0.001$) lower than that of the MM genotype in Bandur ram lambs, and the NN genotype indicated higher meat tenderness. In the Dorset Down breed, ac genotypes had higher shear force (SF) in fillets from ewes (Palmer et al. 1997). Meat samples from ab showed a higher initial mean pH in the Dorset Down breed and aa reported a higher final pH (after ten days) in the Santa Inês breed (Palmer et al. 1997; Esteves et al. 2020). Jawasreh et al. (2017) reported that MN had lighter meat and MN showed lower cooking loss than MM in Awassi sheep.

In contrast, various studies found no association between the *CAST* gene and different traits (Sutikno et al. 2011; Dehnavi et al. 2012; Nikmard et al. 2012; Peirvisi et al. 2020; Bayram et al. 2019). The available research has shown a significant correlation between *CAST* SNPs and various traits in sheep. It is important to note that the same allele may have different effects in different breeds and therefore breed specific studies are required before using the *CAST* gene as a marker in selection.

4. Conclusions

The *CAST/Msp*I polymorphism was investigated in the Central Anatolian Merino, Pırlak, Romanov and Suffolk sheep breeds by PCR-RFLP method. In this gene region, variations were observed at different frequencies across all three genotypes (MM, MN, and NN) of the CAM sheep breed. Additionally, a high level of genetic diversity was found within the CAM population. Based on these findings, it is proposed that the relevant gene region could be utilized in Marker-Assisted Selection (MAS) studies aimed at enhancing meat yield and quality in the CAM breed. However, prior to initiating MAS studies, it would be prudent to conduct correlation analyses between the identified genotypes and the phenotypic data associated with meat yield and quality.

Acknowledgements

This work has been supported by Eskisehir Osmangazi University Scientific Research Projects Coordination Unit under grant number 2021-230A5. This study is a part of MSc thesis entitled first author.

References

Afanasyeva A, Sarychev V, Goncharenko G (2019) Phenotypic effects of polymorphism of the calpastatin gene (CAST), associated with growth and development indicators, in West Siberian mutton breed. International Scientific and Practical Conference "Digital Agriculture-Development Strategy" 167: 116-120.

Asadi N, Nanekarani S, Khederzadeh S (2014) Genotypic frequency of calpastatin gene in lori sheep by polmerase chain reaction-restriction fragment lengt polymorphism (PCR-RFLP) method. African Journal of Biotechnology 13: 1952-1954.

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

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

Bayraktar M, Shoshin O (2022) Association between CAST and MSTN gene polymorphisms with growth traits in Awassi sheep. Kuwait Journal Science 49(2): 1-15.

Bayram D, Akyüz B, Arslan K, Özdemir F, Aksel EG, Çınar MU, (2019) Akkaraman kuzularında DGAT1, CAST ve IGF-I gen polimorfizmleri ve sütten kesim yaşına kadarki canlı ağırlık artışı üzerine etkileri. Kafkas Üniversitesi Veteriner Fakültesi Dergisi 25(1): 9-15.

Bozhilova-Sakova M, Dimitrova I, Tzonev TI, Petrov N (2020) Genotype frequencies in calpastatin (CAST) and callipyge (CLPG) genes in Northeast Bulgarian Merino sheep breed using PCR-RFLP method. Bulgarian Journal of Agricultural Science 26(2): 475-479.

Chung H, Davis M (2012) PCR-RFLP of the ovine calpastatin gene and its association with growth. Asian Journal of Animal and Veterinary Advances *7*(8): 641-652.

Ciobanu DC, Bastiaansen JWM, Lonergan SM, Thomsen H, Dekkers JCM, Plastow GS, Rothschild MF (2004) New alleles in calpastatin gene are associated with meat quality traits in pigs. Journal of Animal Science 82: 2829-2839.

Dehnavi E, Ahani Azari M, Hasani S, Nassiry MR, Mohajer M, Khan Ahmadi AR, Shahmohamadi L, Yousefi S (2012) Association between yearling weight and calpastatin and calpain loci polymorphism in Iranian Zel sheep. Iranian Journal of Applied Animal Science 2(2): 131-135.

Demir E, Ceccobelli S, Bilginer U, Pasquini M, Attard G, Karsli T (2022) Conservation and selection of genes related to environmental adaptation in native small ruminant breeds: a review. Ruminants 2(2): 255-270.

Esteves C, do Livramento KG, Paiva LV, Peconick AP, Garcia IF, Garbossa CAP, Cardoso TB, Faria PB (2020) Polymorphism of CAST and GDF-8 genes and association with physical-chemical parameters of sheep meat. Genetics and Molecular Research 19(3): 1-12.

Gabor M, Trakovicka A, Miluchova M (2009) Analysis of polymorphism of cast gene and clpg gene in sheep by pcr-rflp method. Scientific Papers Animal Science and Biotechnologies 42: 470-476.

Georgieva S, Hristova D, Dimitrova I, Stancheva N, Bozhilova-Sakova M (2015) Molecular analysis of ovine calpastatin (CAST) and myostatin (MSTN) genes in synthetic population Bulgarian Milk sheep using PCR-RFLP. Journal of BioScience Biotechnology 4(1): 95-99.

Gharahveysi S, Abbasi HA, Irani M, Abdullahpour R, Mirhabibi S (2012) Polymorphism investigation of calpastatin gene in Zel sheep population of Iran by PCR-RFLP method. African Journal Biotechnol 11: 3211-3214.

Goll DE, Thompson VF, Taylor RG, Ouali A (1998) The calpain system and skeletal muscle growth. Canadian Journal of Animal Science 78(4): 503-512.

Gorlov IF, Shirokova NV, Randelin AV, Voronkova VN, Mosolova NI, Zlobina EY, Kolosov YA, Bakoev NF, Leonova MA, Bakoev SY, Leonova MA, Bakoev SY, Kolosov AY, Getmantseva LV (2016) CAST/MspI gene polymorphism and its impact on growth traits of Soviet Merino and Salsk sheep breeds in the South European part of Russia. Turkish Journal of Veterinary and Animal Sciences 40(4):399-405.

Ibrahim AHM, Ismail IM, Shehata MF, El-Beltagy AR (2015) Calpastatin polymorphism in Barki lambs and their effects on growth and carcass traits. Journal of American Science 11(3): 106-112.

Javanmard A, Panandam JM, Sugnaseelan S, Yusoff K (2010) Allele frequencies at six candidate genes associated with growth and carcass quality traits in the Boer goats. African Journal of Biotechnology 9(43): 7236-7238.

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

Jawasreh KI, Al-Amareen AH, Aad PY (2019) Relationships between Hha1 calpastatin gene polymorphism, growth performance, and meat characteristics of Awassi sheep. Animals 9(9): 667.

Jawasreh KI, Ismail ZB (2019) Effects of calpastatin gene polymorphism on hematology and selected serum biochemical parameters in Awassi lambs. Journal of Advanced Veterinary and Animal Research 6(2): 193-196.

Kania GM, Gruszecki TM, Junkuszew A, Juszczuk-Kubiak E, Florek M (2019) Association of CAST gene polymorphism with carcass value and meat quality in two synthetic lines of sheep. Meat Science 154: 69-74.

Karslı T, Balcıoğlu MS, Demir E, Fidan HG, Aslan M, Aktan S, Şahin, E (2017) Determination of polymorphisms in IGF-I and NPY candidate genes associated with egg yield in pure layers chicken lines reared in the Ankara Poultry Research Institute. Turkish Journal of Agriculture-Food Science and Technology 5(9): 1051-1056.

Kawasaki H, Kawashima S (1996) Regulation of the calpain-calpastatin system by membranes. Molecular Membrane Biology 134: 217-224.

Khan SH, Riaz MN, Ghaffar A, Ullah MF (2012) Calpastatin (CAST) gene polymorphism and its association with average daily weight gain in Balkhi and Kajli sheep and Beetal goat breeds. Pakistan Journal of Zoology 44: 377-382.

Knight MI, Daetwyler HD, Hayes BJ, Hayden MJ, Ball AJ, Pethick DW, McDonagh MB (2012) Discovery and trait association of single nucleotide polymorphisms from gene regions of influence on meat tenderness and long-chain omega-3 fatty acid content in Australian lamb. Animal Production Science 52(7): 591.

Kolosov YA, Gorlov IF, Kolosov AY, Shirokova NV, Kulikova AY, Kolosova MA, Slozhenkina MI, Vorontsova ES, Kolosova NN (2021) Determination of CAST gene polymorphism in sheep of the Volgograd breed. *IOP* Conference Series: Earth and Environmental Science 677(5): 052112.

Kumar SN, Jayashankar MR, Ruban W, Sreesujatha RM, Devi YSR (2018) Association of calpastatin polymorphism with carcass and meat quality traits of Bandur sheep. International Journal of Livestock Research 8(5): 232-243.

Nanekarani S, Khederzadeh S, Kaftarkari AM (2011) Genotypic frequency of calpastatin gene in Atabi sheep by PBR method. International Conference on Food Engineering and Biotechnology 9: 189-192.

Nassiry MR, Tahmoures PM, Javad MA, Soltani M, Foroutani FS (2006) Calpastatin polymorphism and its association with daily gain in Kurdi sheep. Iranian Journal of Biotechnology 4(3): 188-192.

NCBI (2021) National Center for Biotechnology Information, CAST calpastatin [Ovis aries (sheep)], https://www.ncbi.nlm.nih.gov/gene/?term=443364, Accessed 28 December, 2021.

Nikmard M, Molaee V, Eskandarinasab MP, Dinparast Djadid N, Vajhi AR (2012) Calpastatin polymorphism in Afshari sheep and its possible correlation with growth and carcass traits. Journal of Applied Animal Research 40(4): 346-350.

Palmer BR, Roberts N, Kent MP, Ilian MA, Le Couteur C, Morton J, Hickford JGH, Bickerstaffe R (1997) Calpastatin as a candidate gene for sheep meat tenderness. Annual International Congress of Meat Science and Technology 43: 590-591.

Palmer BR, Roberts N, Hickford JG, Bickerstaffe R (1998) Rapid communication: PCR-RFLP for MspI and NcoI in the ovine calpastatin gene. Journal of Animal Science 76: 1499-1500.

Palmer BR, Morton JD, Roberts N, Ilian MA, Bickerstaffe R (1999) Marker–assisted selection for meat quality and the ovine calpastatin

gene. Proceedings of the New Zealand Society of Animal Production 59: 266-268.

Peirvisi N, Noshary A, Hemati B (2020) The calpastatin gene polymorphism study and its effect on early body weight traits in Zandi sheep. Iranian Journal of Applied Animal Science 10(2): 297-302.

Pomitun IA, Rossokha VI, Boyko YA, Guzevatyi OE, Shpilka MV, Kulibaba RO (2019) Analysis of calpastatin and callipyge genes polymorphism in Prydniprovska meat sheep. Agricultural Science and Practice 6(2): 58-65.

Ramadevi B, Kumari BP, Sudhakar K, Gangaraju G, Vinod U (2020) Polymorphism of the ovine calpastatin (CAST) gene and its association with productive traits in Nellore sheep. Journal of Animal Research 10(6): 881-887.

Ropka-Molik K, Bereta A, Tyra M, Różycki M, Piórkowska K, Szyndler-Nędza M, Szmatoła T (2014) Association of calpastatin gene polymorphisms and meat quality traits in pig. Meat Science 97(2): 143-150.

Selionova MI, Chizhova LN, Surzhikova ES, Podkorytov NA, Podkorytov AT, Voblikova TV (2020) Meat productivity of sheep of the Altai Mountain breed of different genotypes according to the CAST and GDF9 genes. IOP Conference Series: Earth and Environmental Science 613(1): 012130.

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

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

Sunilkumar MC, Nagaraja M, Jayashankar N, Fairoze B, Veeregowda B (2014) Molecular studies on meat quality gene in Bandur sheep. Journal of Cell and Tissue Research 141: 40-49.

Sutikno M, Yamin M, Sumantri C (2011) Association of polymorphisms calpastatin gene with body weight of Local sheep in Jonggol, Indonesia. Media Peternakan 34(1): 1-6.

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

Tohidi R (2013) Molecular analysis of ovine calpastatin gene in six Iranian sheep breeds using PCR-RFLP. Journal of Animal Production Advances 3: 271-277.

Valencia CPL, Franco LÁÁ, Herrera DH (2022) Association of single nucleotide polymorphisms in the CAPN, CAST, LEP, GH, and IGF-1 genes with growth parameters and ultrasound characteristics of the Longissimus dorsi muscle in Colombian hair sheep. Tropical Animal Health and Production 54(1): 82.

Yeh FC, Yang RC, Boyle TBJ, Ye ZH, Mao JX (1997) Popgene, The user-friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Canada.

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

Yılmaz O, Cemal İ, Karaca O, Ata N (2014b) Association of calpastatin (CAST) gene polymorphism with weaning weight and ultrasonic measurements of loin eye muscle in Kıvırcık lambs. Kafkas Üniversitesi Veteriner Fakültesi Dergisi 20: 675-680.

*Ethical approval and permission for this study was obtained from Eskisehir Osmangazi University Animal Experiments Local Ethics Committee (Date: 09/10/2020; Decision No: 809).