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CAST gene effect on body condition score and some ultrasonographic measurements in Karacabey Merino sheep

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Abstract

The calpastatin (*CAST*) gene is one of the candidate genes affecting muscle growth and meat quality. In this study, the effects of the *CAST* gene on backfat thickness (BFT), lumbar muscle depth (Musculus longissimus thoracis et lumborum-MLD) determined by ultrasonic measurements, body condition score (BCS), live weight (LW) were investigated in 200 Karacabey Merino sheep. It was observed that *the CAST* gene allele was more common than the N allele in Karacabey Merino sheep. MM, MN, and NN genotypes were determined as 47.50%, 40.00%, and 12.50%, respectively. It was determined that there was a statistically significant difference between age groups in terms of BFT values, which is one of the ultrasound criteria ($P<0.05$), and there was no significant difference in terms of muscle depth (MD). It was found that gender and genotypes had a significant effect on MD ($P<0.05$) and BFT ($P<0.01$). It was determined that there was a statistically highly significant difference ($P<0.01$) in terms of BCS and LW values between genders and age groups. There was a statistically significant difference ($P<0.05$) between genotypes regarding BCS values; no significant difference was found regarding LW values. The results show that the *CAST* gene effectively affects BFT, MLD muscle depth, BCS, and LW.

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Introduction

Lamb meat is highly ranked among red meats in Turkey. Sheep breeding, essential in animal husbandry, produces raw materials for the textile and dairy sector and provides valuable lamb meat. Due to the interest in the consumption of lamb meat, its excellent culinary value, and its ability to adapt to growing in different climatic and geographical conditions, fattening sheep and sheep meat production has always been the focus of attention in animal production and continues to increase worldwide. With this interest in sheep breeding and the development of animal breeding and feeding techniques, significant progress has been made regarding rapid live weight gain and productivity in livestock (1-4). For this purpose, Karacabey Merino was developed by crossing with German meat Fleece Merino×Kıvırcık to increase wool and meat performance (5). However, at this point, contrary to the increase in yield, it should be noted that meat quality decreases. The live weight gain, fattening performance, and the meat quality obtained from the animals should be considered (6). Consistently better quality of lamb meat should be the industry's top priority. Therefore, a recent shift in livestock appears to be from increased muscle growth to improved meat quality. The influence of breed and genotype on the quality and composition of meat is known. This revealed the importance of choosing the right breed with high meat quality (7). Meat tenderness, water-holding capacity, meat color, chemical structure, nutritional value, and pH are important criteria in determining meat quality. Meat quality is also affected by environmental factors such as age, gender, ration content before slaughter, carcass temperature after slaughter, pH change, maturation, and applied technological processes (8-11). It is a complex system regulated by many genes on body weight gain and

carcass quality. The calpain-calpastatin system is a complex system that is effective in skeletal muscle formation and growth, as well as in meat formation and quality after slaughter (12,13). While calpain is effective in the breakdown of muscle proteins, calpastatin is a calpain-protease inhibitor. Increased calpastatin activity causes a decrease in calpain activity. High calpastatin activates skeletal muscle formation and growth (1,14-20). At the same time, it is effective in determining the birth weight and daily live weight gain of lambs (4,21). It is effective in meat formation, maturation, and tenderness post-slaughter (4,13,21).

Calpastatin (*CAST*) is a gene that affects meat quality such as *Musculus longissimus dorsi* (MLD-low back muscle) area, marbleization degree, fat ratio and back fat thickness, carcass weight, and carcass yield, water holding capacity of meat, tenderness (2,21-23). *The CAST* gene is the candidate gene that is thought to be effective on growth and carcass characteristics on the fifth chromosome in sheep (24-26). Body condition score (BCS) is a subjective measure of fattening performance in live animals. However, it is closely related to body composition and is a simple, functional, and helpful indicator (27). Studies have shown a linear relationship between BCS and live weight gain. On the other hand, there is a linear relationship between BCS and MLD area expansion and back fat thickness. As a matter of fact, while determining the BCS, the back of the sheep is scored by controlling the MLD muscle width (28,29). Using ultrasound, carcass structure, and quality can be determined in live animals. Although ultrasound technology is widely used for reproduction measurements, MLD can measure muscle depth and back fat thickness. In this way, carcass quality can be determined quickly and economically without harming the animal (30-34).

This study investigated the effect of the *CAST* gene on body weight, body condition score (BCS), and some ultrasonic measurements (backfat thickness and MLD muscle depth) in the Musculus Longissimus dorsi-MLD.

Materials and Methods

Ethical Statement

The study was conducted with the permission of the Balikesir University Animal Experiments Local Ethics Committee dated 25.04.2024 and numbered 2024/4-12.

Animal material

The study's animal material consisted of 200 Karacabey Merino lambs, including 24 males and 30 females aged 6 months, 15 males and 20 females aged 8 months, and 11 males and 100 females aged 12 months. Separate groups have been created for each age. Sheep are fed ad libitum roughage and additionally concentrated feed in amounts calculated according to age.

Blood samples collection

Blood samples were collected from the jugular vein, and 10 ml of each was filled into a sterile vacuum tube containing K3-EDTA in a controlled manner. The tube was stored at -20 °C until use.

DNA isolation from blood samples

The DNA isolation was performed at Adnan Menderes University, Faculty of Agriculture, Department of Animal Science, Genetics Laboratory, Aydın.DNA required for the study was isolated from blood samples using a commercial isolation kit (Applied Biological Materials Column-Pure Blood Genomic DNA Kit, Canada). The quantity and quality of DNA samples obtained from the isolation were checked with NanoDrop 2000 (Thermo Scientific, USA).

DNA Amplification by PCR

Genotypes for the*CAST* gene were determined by PCR-RFLP method using the primer pair reported by Khederzadeh (2011) (*CAST* F: 5'-CCTTGTCATCAGACTTCACC-3', *CAST* R: 5'-ACT GAG CTT TTA AAG CCT CT-3'). For the Polymerase Chain Reaction with a total volume of 25 µl, a PCR mixture containing dNTP (0.2mM), MgCl2 (2.0mM), primers (0.25 µM), PCR buffer (1X), and Taq DNA polymerase and 100 ng Genomic DNA and ddH_2O was created. The PCR program used in the thermal converter to amplify primer-specific DNA regions is given in table 1. MspI enzyme was used to cut the amplified PCR products. Enzyme digestion was performed at 37°C for 6 hours and in a 30 µL reaction solution containing 0.50 µL of ddH2O, 3.00 µl of 10X Buffer Tango, 1.50 µLMspI and 25 µL of PCR product. Genotypes were determined by separating and visualizing the cut DNA fragments on a 2% agarose gel.

Table 1: PCR program used in thermal cycler

Steps	ºC	Minute	Cycle
Denaturation	95	2	1
Denaturation	95	1	
Annealing	65	1	35
Extension	72	2	
Final Extension	72	10	1

Determination of live weight and body condition score

Live weights were determined using an electronic scale (Uzay Baskül- UZE-P 300) with a 50 g sensitivity. Body condition score was scored from 1 to 5 (1: Emaciated, 2: Thin, 3: Average, 4: Fat, 5: Obese) (35). It was carried out immediately after the live weight control.

Ultrasonic measurements

Shearing removed The wool from the measurement areas to improve ultrasound image acquisition. Measurements of the characteristics of the MLD were performed with an

ultrasound device (SIUI CTS900V) using a linear probe (8-Mhz) with a scanning area of 6 cm in the region between the 12th and 13th ribs. In addition, liquid ultrasound gel was applied to the scanning area to improve the transmission between the skin and the transducer. In the measurements, the BFT and MD of the MLD muscle were determined as ultrasound criteria (Figure 1).

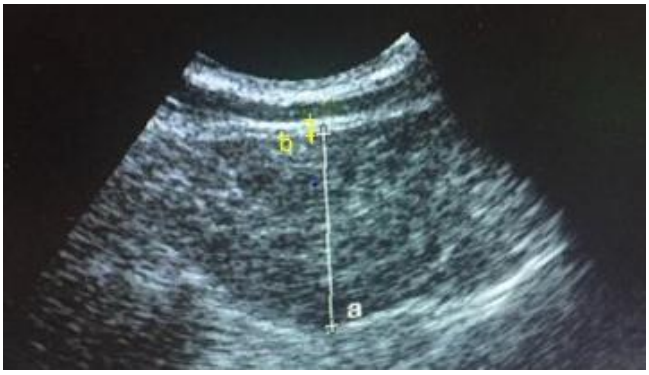

Figure 1: Measured properties a- Muscle Depth (MD), b-Backfat Thickness (BFT)

Statistical analysis

Allele frequencies, genotype frequencies, and chi-square ($\chi 2$) tests were obtained using GenAlEx (36,37) and Popgene32 (38) programs. The GLM procedure of the SAS (39) package statistics program was used for variance analysis to determine the change in body weight, body condition score, and ultrasonic measurement data according to *CAST* genotypes. DUNCAN was used for the multiple comparison tests (39).

Results

A statistically significant difference (P<0.05) between age groups regarding BFT values among ultrasound criteria was found. There was no statistically significant difference in MD between age groups. It was determined that there was a statistically highly significant difference (P<0.01) between age groups in terms of BCS and live LW values. It was found that there was a statistically significant difference between genders in terms of MD (P<0.05), BFT (P<0.01), BCS, and LW values. Genotypes were found to have a highly significant effect on MD and BFT (P<0.01). It was determined that there was a statistically significant difference (P<0.05) between genotypes in terms of BCS values. There was no statistically significant difference between genotypes in terms of LW values. LW had a significant effect on ultrasound measurement parameters and BCS. Table 2 gives the mean and standard errors of least squares obtained for live weight, ultrasound measurements, and body condition scores of lambs in different age groups.

Table 2: Least squares mean and standard errors (SE) for body weight, ultrasound measurements, and body condition score

Factors	N	MD	BFT	BCS	LW
Age Group		P=0,432	P=0.049	P=0.008	P=0.000
6th months	54	2,35±0,059	0,13±0,012^{a}	2,85±0,038^{a}	40,64±0,922^{a}
8th months	35	2,25±0,062	0,11±0,013^{ab}	2,69±0,040^{a}	49,05±1,198^{b}
12th months	111	2,28±0,061	0,08±0,013^{b}	2,69±0,039^{b}	61,67±0,860^{c}
Gender		P=0,010	P=0.027	P=0.000	P=0.000
Male	50	2,18±0,06	0,09±0,012	2,58±0,039	58,16±1,019
Female	150	2,4±0,045	0,13±0,009	2,90±0,029	42,74±0,690
CAST Genotype		P=0,000	P=0,000	P=0,039	P=0.199
MM	95	2,45±0,036^{a}	0,14±0,007^{a}	2,81±0,046^{a}	50,64±0,696^{a}
MN	80	2,27±0,044^{a}	0,11±0,009^{a}	2,74±0,023^{a}	51,71±0,860^{b}
NN	25	2,15±0,071^{b}	0,07±0,015^{b}	2,68±0,029^{b}	49,01±1,383^{c}
Reg (Linear)		P=0.000	P=0.000	P=0.000	
LW		0,019±0,004	0,004±0,001	0,018±0,002	
Overall	200	2,29±0,033	0,11±0,007	2,74±0,021	50,45±0,647

MD: Muscle depth, BFT: backfat thickness, BCS: Body Condition score, LW: Live weight.

DNA bands formed due to PCR-RFLP were visualized by separating them in 2% agarose gel (Figure 2). For the separation of the M and N alleles of the Calpastatin gene, the 565 bp long PCR product amplified with the primer pairs was cut with restriction enzyme (MspI). Due to a single point mutation occurring in the cut region of the enzyme, this cut region has disappeared in some individuals, and no cuts have been made. Therefore, only a single band with a length of 565 base pairs was observed in individuals exhibiting this condition, and these individuals were genotyped as NN. Individuals showing three bands of 565, 306, and 259 base pairs were genotyped as MN, while individuals with two bands with a length of 306 and 259 base pairs were genotyped as MM due to a mutation in only one of the alleles.

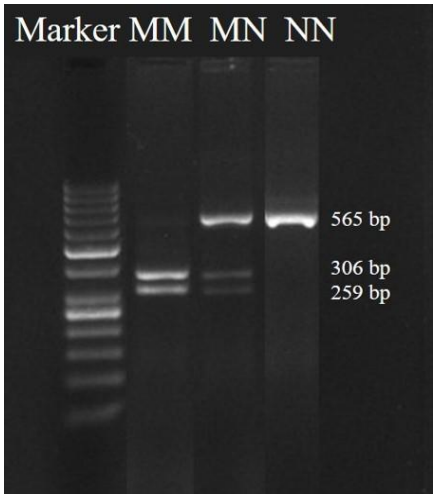

Figure 2: Genotyping of *the CAST* gene by Restriction Fragment Length Polymorphism (PCR-RFLP) method.

Table 3 gives allele frequencies, genotype frequencies, observed (Ho) and expected heterozygosity (He) values obtained from the study, and chi-square test results for Hardy-Weinberg equilibrium. The frequency of M and N alleles in Karacabey Merino sheep was 67.50% and 32.50%, respectively. Accordingly, it showed that the M allele is more common in populations than the N allele. Considering the genotype frequencies, all three MM, MN, and NN genotypes were observed in the Karacabey Merino breed. MM, MN, and NN genotype frequencies were found to be 47.50%, 40.00%, and 12.5%, respectively. The genotype frequency of MM genotypes was found to be relatively higher than the others (47.50%). Observed heterozygosity (Ho) and expected heterozygosity (He) values of the *CAST* gene were found to be 0.400 and 0.439, respectively. It was also observed that the population analyzed for this gene was not in Hardy-Weinberg equilibrium.

Table 3: Allele, genotype frequencies observed (Ho) and expected (He) heterozygosity in terms of CAST gene in Karacabey Merino lambs, Chi-square test ($\chi 2$)

Locus	N	Allele Freq (%)		Genotype Freq (%)			Heterozygosity		
		M	N	MM	MN	NN	Ho	He	χ^2
CAST	200	67.50	32.50	47.50	40.00	12.50	0.400	0.439	1.560^{ns}

ns: nonsignificant.

Discussion

Determining BCS is easy and practical for monitoring fattening performance. Lagonikou *et al.* stated that BCS could be considered an alternative in their study on estimating carcass characteristics with ultrasound measurements. However, they stated that the evaluator's experience and performance in determining the correct score should be addressed when determining BCS (34). It was determined that there was a statistically significant difference between the genotypes in terms of BCS values. As in the ultrasound measurements, it was observed that lambs with MM and MN genotypes showed better performance in terms of BCS, while those with NN genotypes showed poor performance. Therefore, the *CAST* gene significantly affects BCS.

It is observed that ultrasound measurements (MD and BFT) and BCS decrease with age. This can be attributed to the fact that the performance of 6-month-old lambs was better because they suckled milk and were also given additional feed, while the 8-and 12-month-old lambs were only fed adlibitum. Considering the genders, as expected, ultrasound measurements (MD and BFT), BCS, and LW showed higher performance in male lambs than females. These results are consistent with literature data (31-33).

Calpastatin is one of the genes on which studies are intense. It is seen that these studies focused on the effects of the calpastatin gene on MLD muscle depth, backfat thickness criteria determined by ultrasonic measurements, and phenotypic features such as live weight, live weight gain, age, weaning age, and weight. When the literature is reviewed, it is stated that the *CAST* gene is more common in the population than the N allele of the M allele. In some populations, the frequency of the N allele is low; even populations that do not have the N allele have been reported. Dimitrova *et al.* In his study on Bulgarian sheep, the N allele was not found in Cooper-Red Shumen sheep (20). The allele N was not found in a study on Lacaune and Eastern Frisian breeds (21,40). While the N allele frequency in the three regions varied between 0.07% and 0.11%, the N allele was not found in the fourth region (3). Avanus found the highest M allele frequency in some Turkish native sheep breeds in İmroz 96%, and the lowest N allele frequency in Kıvırcık sheep breeds (41). In Karacabey Merino lambs, the allele frequency was determined as M 67.5% and N 32.5%. Asadi *et al.* show parallelism with M 63.8% and N 36.2% allele frequencies in Lori sheep (42). Yılmaz *et al.* found the allele frequency of Karacabey Merino as M 80.04% and N 19.96% in their study (40).

MM, MN, and NN genotypes of the Karacabey Merino *CAST* gene were observed. Genotype frequencies were determined as 47.50%, 40.00%, and 12.50%, respectively. Szkudlarek-Kowalczyk *et al.* determined the genotype frequencies in Polish Merino sheep as 56.10%, 40.10%, and

3.70%, respectively (43). In particular, the MN genotype appears to be the same. The genotype frequency of Lory sheep is 40.70%, 46.20%, and 13.10%, respectively (31). Ata and Cemal found the genotype frequency in Karya sheep to be 56.30%, 38.8%, and 6.90%, respectively (44). Yılmaz *et al.* found the Karacabey Merino genotype frequencies to be 66.94, 26.21 and 6.85%, respectively (31). Avanus, the highest genotype frequencies were determined in MN Kıvırcık 60%, MM İmroz 92.6%, and NN Morkaraman 7.1% sheep breeds. However, the NN genotype was not found in Kıvırcık, İmroz, Karayaka, and Karagül breeds (41). Studies have reported that sheep with the M allele of the *CAST* gene show higher performance than those with the N allele, and MM and MN genotypes show higher performance than NN genotypes. It is seen that the Karacabey Merino M allele 67.50% is high. Although MM 47.50% and MN 40.00% genotype frequencies are relatively low, considering that both genotypes are associated with high yields, it can be said that they are high overall in terms of genotype frequencies.

Genotypes also appear to have a significant impact on ultrasound measurements. It is observed that lambs with MM and MN genotypes perform better in terms of muscle depth and backfat thickness (BFT). Those with the NN genotype showed poor performance. In parallel with the literature, selection is in the direction of MM and MN genotypes. Valencia *et al.* reported in their study that the *CAST* gene significantly affects birth weight in lambs, but it does not show a significant difference in MD and BFT (16). In their study on Kıvırcık lambs, Yılmaz *et al.* investigated the effects of the *CAST* gene, which is important for meat quality, on weaning weight, average daily gain, and MLD traits. They reported that C*AST* alleles particularly affect BFT and S+BFT and have fewer fat carcasses than lambs with the NN genotype (30). Cemal *et al.* reported in their study on genetic prediction from genetic parameters related to ultrasound measurements in Kıvırcık lambs that BFT, S+BFT, and MD are significantly affected, while LW is less affected. They stated that BFT and Skin+BFT can be used as selection criteria (33). The effect of other genes affecting meat yield and carcass quality on BCS and ultrasonic measurements should be investigated. This will contribute significantly to genomic selection studies.

Conclusion

With live weight, BCS, and ultrasonic measurements, it is possible to determine lambs' fattening performance and meat quality without slaughtering them at any age. The study shows that the *CAST* gene significantly affects body weight, BCS, and some ultrasonic measurements.

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Conflicts of interest

The authors declare no conflicts of interest.

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تأثير جين كالباستاتين على درجة حالة الجسم وبعض القياسات بالموجات فوق الصوتية في أغنام كاراجابي ميرينو

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الخلاصة

جين كالباستاتين (CAST) هو أحد الجينات المرشحة التي تؤثر على نمو العضلات وجودة اللحوم. في هذه الدراسة، تم التحقيق في تأثيرات جين CAST على سماكة الدهون الخلفية، وعمق العضلات القطنية التي تم تحديدها بواسطة القياسات بالموجات فوق الصوتية، ودرجة حالة الجسم ، والوزن الحي في ٢٠٠ من أغنام المارينو. لوحظ أن أليل الجين CAST كان أكثر شيوعا من أليل N في أغنام المارينو. تم تحديد الأنماط الجينية MM و MN و NN على أنها ٤٧,٥٠٪ و ٤٠,٠٠٪ و ١٢,٥٠٪ على التوالي. تبين أن هناك فرقا معتدا به إحصائيا بين الفئات العمرية من حيث قيم سماكة الدهون الخلفية، وهو أحد معايير الموجات فوق الصوتية ($P<0.05$)، ولم يكن هناك فرق كبير من حيث عمق العضلات. وجد أن الجنس والأنماط الجينية كان لهما تأثير كبير على عمق العضلات ($P<0.05$) و سماكة الدهون الخلفية ($P<0.01$). تقرر أن هناك فرقا كبيرا من الناحية الإحصائية ($P<0.01$) من حيث قيم درجة حالة الجسم والوزن الحي بين الجنسين والفئات العمرية. كان هناك فرق ذو دلالة إحصائية ($P<0.05$) بين الأنماط الجينية فيما يتعلق بقيم درجة حالة الجسم. لم يتم العثور على فرق يعتد به فيما يتعلق بقيم الوزن الحي. كما أظهرت النتائج أن جين CAST يؤثر بشكل فعال على سماكة الدهون الخلفية و وعمق العضلات القطنية ودرجة حالة الجسم والوزن الحي.