

Genotypic characterization of meat-type lambs expressing the *callipyge* gene in Turkey: I. Carcass characteristics and retail yield

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Received: 04.12.2021

Accepted/Published Online: 24.01.2022

Final Version: 23.02.2022

Abstract: This study is set out to evaluate the effects of *callipyge* (*CLPG*) gene, attributed to sheep affecting growth and meat quality, on carcass and sensory trait of most preferred meat-type lamb (Kıvrıkcık, Karacabey Merino, Ramlıç, German Black-Head Mutton × Kıvrıkcık, Hampshire Down × Merino) in the western part of Turkey. Two datasets were used: (i) 177 lambs (66 males, 111 females) to determine genotype and allele frequencies; (ii) 48 genotypically identified male lambs for the assessment of carcass and meat sensory quality. It was found that homozygous (*NN*) and heterozygous (*MN*) conditions of the *CLPG* gene were observed in all sheep breeds, except Ramlıç which exhibits only *NN*. No significant differences were observed in *CLPG* genotypes for slaughter weight, cold carcass weight, and cold dressing percentage ($p > 0.05$). On the other hand, the effect of genotype on neck percentage was significant ($p < 0.01$), and lambs expressing *CLPG* genotype (*MN*) had a higher neck percentage. Not significantly, but numerically the *MN* genotype had a higher percentage of the shoulder, rack, and leg than the *NN* genotype, while the *NN* genotype had a higher percentage of loin ($p > 0.05$). Similarly, the effect of genotype on meat quality assessment was not significant ($p > 0.05$); however, shear force, water holding capacity, and cooking loss of the *NN* genotype were higher than the *MN* genotype. Indeed, no interaction between genotype and time was observed on the color parameter of the *Longissimus thoracis et lumborum* muscle at various storage periods ($p > 0.05$). The findings suggest that all meat-type sheep breeds (except Ramlıç) were polymorphic, suggesting that heterozygous individuals who received the *CLPG* mutation from the sire should be adopted in selection programs to improve carcass traits.

Key words: *Callipyge*, lamb, primal cuts, carcass, shear force, meat quality

1. Introduction

Identification and localization of genes responsible for the economically important traits in livestock and applying beneficial alleles in selection programs is a major area of interest within the field of molecular genetics [1]. A key aspect of using genetic markers in selection programs is the acceleration of animal growth, meat yield, and meat quality [2,3].

As it has already been noted, one of the most well-known major genes connected to sheep affecting growth and meat quality characteristics is *callipyge* (*CLPG*), located on the distal chromosome 18, which causes postnatal muscle hypertrophy in paternal heterozygous lamb's hindquarter and loin [4–6]. Lambs, having the *CLPG* phenotype, are characterized by a better feed conversion, higher leg scores, superior dressing percentage, preferable lean composition, and firmness of meat due to increased

calpastatin level, which inhibits μ -calpain activity and prevents meat tenderization in time [3,7,8]. Ma et al. [9] also stated that reduced body fat accretion and type IIB muscle fiber occurrence are observed only when a paternally inherited *CLPG* mutation allele combines with a normal maternal allele.

A potential advantage of using *CLPG* mutated lamb in meat production systems is its significant effect on decreasing the cost of lamb profitability, as stated earlier by Busboom et al. [10]. On the other hand, one of the main difficulties with this line of reasoning is accepting these *CLPG* mutated lamb meat by packers and consumers and adopting their breeding scheme for improved lamb meat production [10,11]. To our best knowledge, before this investigation, there were no data on the genotypic characterization of various meat-type lambs expressing the *CLPG* gene raised in Turkey. Therefore, the objective of

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this study is to focus on the characterization most preferred meat-type lamb (Kıvırcık, K; Karacabey Merino, KM; Ramlıç, R; German Black-Head Mutton × Kıvırcık, GBK; Hampshire Down × Merino, HM) by producers in the western part of Turkey and define the relationship between *CLPG* genotypes and retail carcass parts considering the consumers and market's demands.

2. Materials and methods

The procedures of this study were approved by the Ethical Committee of the Sheep Breeding Research Institute, Balıkesir, Turkey (approval number: 13360037), and the experiments were conducted according to the guidelines of the Declaration of Helsinki over the lambing season of 2018 from January to June in the same Institute's experimental farm unit.

2.1. Animal selection and feeding regimen

The details of animal background and feeding regimen are provided by Kader Esen et al. [12] and Kader Esen & Elmacı [13]. Briefly, a total of 202 lambs were selected from five different meat-type sheep breeds (15 ♂ and 36 ♀ of Kıvırcık, 14 ♂ and 33 ♀ of Karacabey Merino, 14 ♂ and 14 ♀ of Ramlıç, 15 ♂ and 34 ♀ German Black-Head Mutton × Kıvırcık, 11 ♂ and 16 ♀ of Hampshire Down × Merino crossbreed) raised in Sheep Breeding Research Institute to create a single flock after the weaning of lambs at an average age of 90 ± 6 days. Lambs have suckled their dams up to their weaned, and from 15 days of age, they were free to access commercial starter feed and high-quality alfalfa hay *ad libitum*. Each lamb had received an average of 600 g of concentrate feed, 100 g of high-quality alfalfa hay, and 300 g of vetches-wheat mixtures hay per day until the slaughtering period.

2.2. Blood sampling and DNA isolation

Blood samples were taken from the *vena jugularis* of each lamb into sterile EDTA tubes, and genomic DNA was isolated from blood by using a GeneAll® DNA extraction kit. Purified genomic DNA was amplified by using Bio-Rad T100 thermal cyclers. PCR amplification of 214 bp DNA fragment of *CLPG* locus was amplified using primers F: 5'-GGAATCATCGTGTCTGGTC-3' and R: 5'-CCAGCAGGATACTCCGGTC-3' [14]. The PCR amplifications were carried out with 30 µL reaction solution containing 50 ng the purified genomic DNA, 11.0 µL nuclease-free sterile water, 1.0 µL of each primer (forward and reverse primer), 2x Amp Master™ Taq (GeneAll; contain 2.5 U Taq DNA polymerase, dNTP 200 µM, reaction buffer 1x, loading dye & stabilizer 1x). The cycling protocol was 2 min at 95 °C for initial denaturation, 35 cycles of amplification; 95 °C for 20 s, 58 °C annealings for 30 s, 72 °C for the 60s and 4 min at 72 °C for the final extension. Afterward, 10 µL of PCR product was digested with *AvaII* (Thermo Scientific) restriction

endonuclease enzymes and incubated at 37 °C for 1 h to run on 3.0% agarose gel containing Syber Safe DNA Stain. PCR products were visualized under UV light in the Gel Documentation System.

2.3. Slaughter and sample collection

A total of 50 ♂ lambs (10 of each breed) were randomly selected to investigate carcass measurements and yields. Selected lambs were fasted for 12 h in Institute's slaughterhouse with free access to fresh water and then slaughtered according to commercial procedures. The slaughter weight (SW) of each lamb was recorded immediately before slaughter. The cold carcass weight (CCW) was recorded after chilling carcasses at 4 °C for 24 h, and the cold dressing percentage was calculated from the ratio of CCW to SW. Afterward, each carcass was separated into 5 primal cuts (neck, shoulder, rack, loin, and leg) and weighed [12]. The *Longissimus thoracis et lumborum* (LTL) muscle from the 5th to 12th thoracic vertebrae on the right-half carcass was taken for further laboratory analyses.

2.4. Meat quality assessment

The color of LTL was measured using a colorimeter (Chroma Meter CR-410; Konica Minolta, Tokyo, Japan) after calibrating a white tile using a D 65 illuminant and observer angle of 10° [15]. The lightness (*L*), redness (*a*), yellowness (*b*), chroma (*C*), and hue (*h*) values were instrumentally obtained from an average of three measurements for each sample after the storage of 0, 48, and 168h at 4 °C. Water holding capacity (WHC) was quantified using the filter-paper method as previously described by Honikel & Hamm [16]. The sample of LTL was analyzed for thawing loss (TL) and cooking loss (CL), as previously reported by Choi et al. [17] and Gonzales-Barron et al. [18], respectively. Shear force (SF) of LTL was analyzed using the cooked samples (1 cm × 1 cm × 2 cm) in the Central Research Laboratory of Namık Kemal University, Tekirdağ, Turkey. The SF was measured using a TA.HDplus Texture Analyser (Stable Micro Systems Ltd., Surrey, UK) and an HDP/WBV Warner Bratzler Blade. The mean maximal cutting strength of cooked LTL samples was obtained from three technical replicates.

2.5. Statistical analysis

The direct counting method was used to calculate the allelic and genotypic frequencies, expected, and observed heterozygosity using Popgene version 1.32 software [19]. The chi-square test (χ^2) was used to determine the population in Hardy-Weinberg equilibrium. The effect of *CLPG* genotypes on carcass traits and meat quality was assessed using the multifactor analysis of variance using the least-squares (LS) method of Minitab [20]. Repeated-measures analysis of variance was used to test for statistically significant differences for color parameters. The models used for LS analysis of carcass traits and meat quality were as follows:

$$Y_{ijklm} = \mu + a_i + b_j + c_k + d_l + e_{ijklm}$$

Where : the observation of the mth animal within the lth genotype of the kth age group of the jth birth type of the ith breed; μ : overall mean; a_i : fixed effect of the ith breed (i: K, KM, R, GBK, HM); b_j : fixed effect of the jth birth type (single, twin); c_k : fixed effect of the dam kth age group (2, 3, 4, 5, 6, 7+); d_l : effect of the lth genotype (j: MN, NN), e_{ijklm} : residual error.

3. Results

Twenty-five of the 202 lambs were excluded from the study due to unidentified genotyping for *CLPG* locus, and two of them also belonged to slaughtered ones.

The digestion of 214 bp PCR product for *CLPG* gene with restriction endonuclease *AvaII* differentiated into M and N alleles. The *AvaII* digestion of the PCR products produced digestion fragments of 214 bp, 137 bp, 77 bp for heterozygous MN genotype and 137 bp, 77 bp for homozygous NN genotype (Figure 1). The homozygous MM genotype (undigested fragment of 214 bp) was not seen in these meat-type lamb populations. Closer inspection of Table shows the genotypic and allelic frequency of *CLPG* genes, represented by two alleles M and N; in this gene, N is more frequent than M and the percentage frequencies are 96.89 and 3.11 in the overall lamb population, respectively. The most important result was that higher MN genotype frequency was observed in the indigenous K breed and its crossbreeds (GBK and KM) as 12.50%, 6.25%, and 4.88%, respectively.

Looking at Figure 2, it is apparent that no significant differences were observed in *CLPG* genotypes for SW, CCW, and CDP ($p > 0.05$). However, the MN genotypes had a greater weight at 7.62% and 3.52% than NN for SW and CCW, respectively. Besides, the NN genotype had a higher dressing percentage at 4.38% than MN genotypes for CDP.

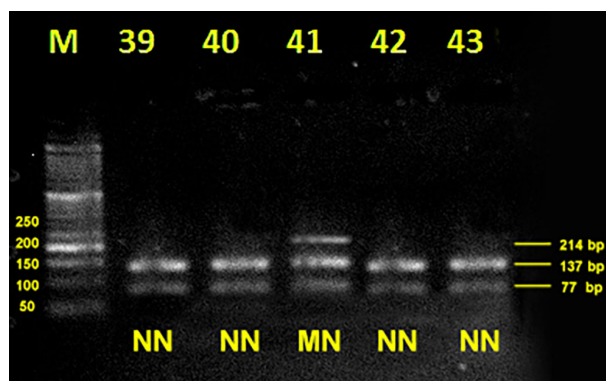


Figure 1. Band patterns of PCR-RFLP by digesting 214 bp DNA fragment of *CLPG* gene with the use of *AvaII* restriction endonuclease enzymes. The MN genotype presents three fragments (214 bp, 137 bp, 77 bp), while NN presents two fragments (137 bp, 77 bp).

The retail carcass percentage of *CLPG* genotypes is illustrated in Figure 3. One unexpected finding was the significant association between the *CLPG* gene and the neck percentage. The MN genotype had a 28.40% higher neck percentage than the NN genotype ($p < 0.01$). Not significantly, but numerically, the MN genotype had a higher percentage of 1.79% shoulder, 9.15% rack, and 4.28% leg than the NN genotype, while the NN genotype had a higher percentage of 2.83% loins than the MN genotype ($p > 0.05$).

Figure 4 compares the meat quality assessment of *CLPG* genotypes in meat-type lambs. What stands out in this figure is that SE, WHC, and CL of the NN genotype were 5.01%, 12.24%, and 10.49% higher than the MN genotype, while TL was 34.21% lower ($p > 0.05$).

An inspection of the data in Figure 5 reveals that no significant interactions between genotype and time

Table. Allelic and genotypic frequency of *CLPG* gene in meat-type lamb breeds raised in Turkey.

Breed	N	GF (%)			AF (%)		Exp Homo (%)	Exp Het (%)	χ^2	P
		MM	MN	NN	M	N				
K	40	0.00	12.50	87.50	6.25	93.75	88.13	11.87	14.05	0.708
KM	41	0.00	4.88	95.12	2.44	97.56	95.18	4.82	12.66	0.910
R	24	0.00	0.00	100.0	0.00	100.0	100.0	0.00	-	-
GBK	48	0.00	6.25	93.75	3.12	96.88	93.88	6.12	3.30	0.856
HM	24	0.00	4.17	95.83	2.08	97.92	95.89	4.11	0.04	1.000
Overall	177	0.00	6.21	93.79	3.11	96.89	93.96	6.04	16.50	0.685

GF: Genotype frequency, AF: Allele frequency, K: Kıvrıcık, KM: Karacabey Merino, R: Ramlıç, GBK: German Black-Head Mutton × Kıvrıcık, HM: Hampshire Down × Merino.

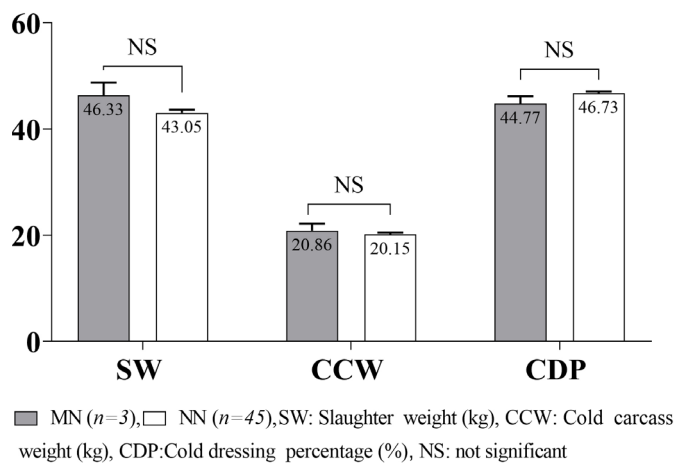


Figure 2. Slaughter weight, carcass weight, and dressing percentage of *CLPG* genotypes in meat-type lambs.

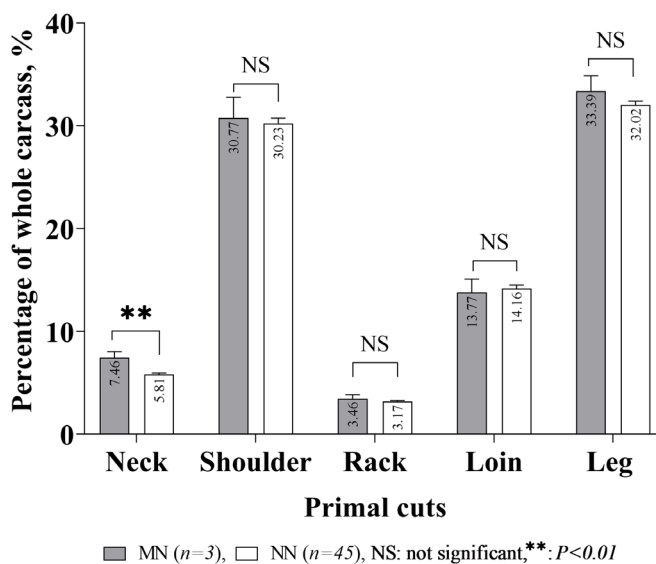


Figure 3. Retail carcass percentage of *CLPG* genotypes in meat-type lambs.

were observed in all color parameters at various storage periods ($p > 0.05$). There is a clear trend of increasing L and h values in both *MN* and *NN* genotypes. After 48 h of storage, a decrease in b and C was detected up to the 168th h. At the end of the storage period, the *MN* genotype had lower L , b , and h values than the *MM* genotype but had higher a and C values.

4. Discussion

Much of the current literature on the *CLPG* gene pays particular attention to the lamb, which has a heavier carcass weight with leaner meat and desirable overall fat content of carcasses among the sheep industry [6,21]. Thus far, many studies have reported the relationship

between *CLPG* genotype and carcass traits, and some of them also include meat sensory characteristics [6,22–24]. Notwithstanding the relatively limited studies about allele and genotype frequency of *CLPG*, this work offers valuable insights into revealing the allele and genotype frequencies with the carcass characteristics of various meat-type sheep breeds at the same time.

In the present study, the genotype and allele frequency of the *CLPG* gene were assessed in the most preferred meat-type lamb in the western part of Turkey. Our results revealed homozygous (*NN*) and heterozygous (*MN*) conditions of the *CLPG* gene in K, KM, GBK, and HM sheep breeds, while R breed was found homozygote (*NN*) for the studied mutation. The results of the current

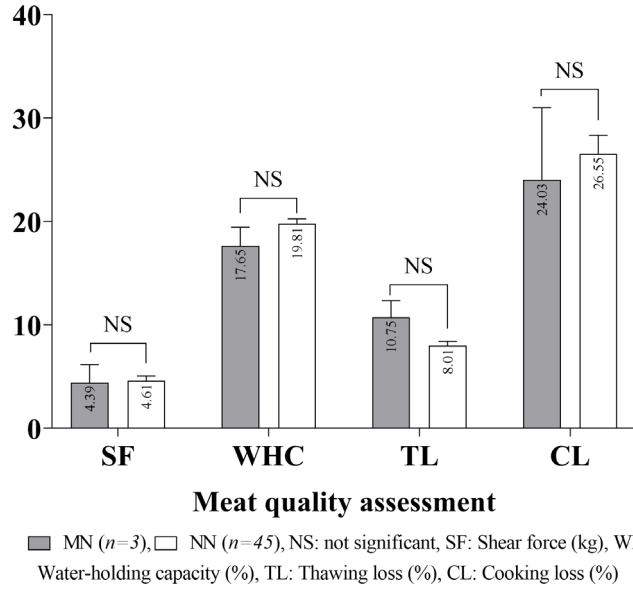


Figure 4. Meat quality assessment of *CLPG* genotypes in meat-type lambs.

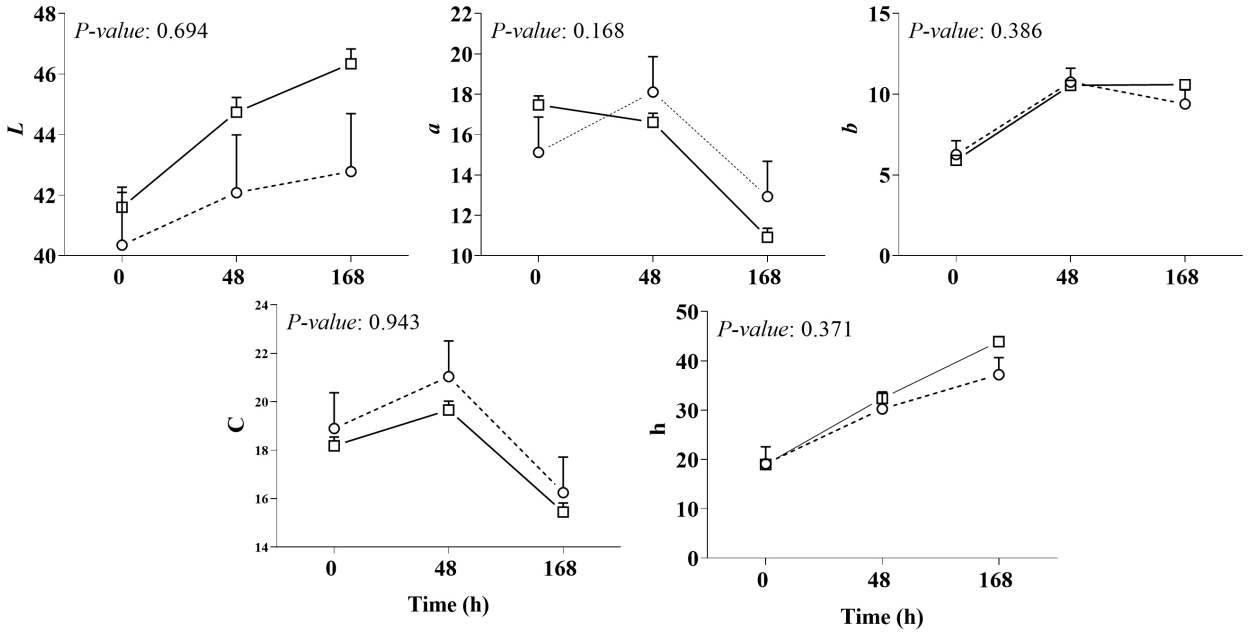


Figure 5. Genotype \times time interaction on color parameters of *Longissimus thoracis et lumborum* muscle during the storage period.

study differ from that of Qanbari et al. [14], Nanekarani & Goodarzi [3], and Othman et al. [5], who reported no mutation in the Afshari and Lori sheep breed in Iran and Barki Rahmani, and Ossimi sheep breeds in Egypt, respectively. Similarly, no mutation was detected in the current study for R lambs. Some sheep breeds in which *CLPG* mutation was not seen were also noted by several

authors, such as Najdi and Harri in Saudi Arabia [25]; Karakachan in Bulgaria [26]; Edilbay, Volgograd, and Kalmyk sheep in the steppe zone of the Russian Federation [27]. However, these results are similar to those reported by Jackson et al. [28] and Shah et al. [29], who found the mutant allele in Dorset, Ramboulee, Hampshire, and Thalli sheep breeds.

It is now well established and confirmed by several authors that lamb carcasses having *CLPG* mutation are more desirable and profitable than normal carcasses [6,23,30]. In the literature, lambs expressing *CLPG* phenotype have been mostly associated with heavier both hot and cold carcass weight and higher dressing percentage than normal muscling lambs [11,28]. Not significantly, but numerically, the current study's findings support the previous research, which indicates *CLPG* mutated lambs had a higher SW and CCW than the normal ones [6,10,31]. However, our results slightly differ for CDP from the results of those authors.

A strong relationship between *CLPG* mutated lamb and carcass primal cuts has been reported in the literature. It was stated that *CLPG* mutated lamb had a higher wholesale leg, loin, rack, and shoulder than normal ones [10,21]. However, there has been no study to record significant differences between *CLPG* genotypes to our best knowledge. The neck percentage of *CLPG* mutated lamb was found to be 28.40% greater than the normal one ($p < 0.01$). On the contrary of our results, Abdulkhaliq et al. [21] stated no significant differences were observed between *CLPG* mutated and normal lambs.

A considerable amount of literature has been published on the relationship between the *CLPG* gene and tenderness. These studies suggest that the reduction rate of protein degradation, correlated with calpastatin activity, and postmortem meat proteolysis, play an essential role in *CLPG* mutated LTL muscle tenderness [24,32,33]. Hence, it could conceivably be hypothesized that an increment of calpastatin activity in *CLPG* mutated lamb results in reduced protein degradation and increased meat toughness [24,34]. These results reflect those of Carpenter et al. [35] and Yu et al. [36], who also stated an increase in the fast-twitch glycolytic fibers and a decrease in slow-oxidative myofibers results in increased *semitendinosus*, *longissimus*, and *gluteus medius* muscle mass. In contrast to earlier findings, however, lower SF values were observed in the mutant *MN* genotype. On the other hand, there is conflicting evidence on the relationship between WHC and *CLPG* mutated LTL muscle. While, Abdulkhaliq et al. [37] reported that higher moisture content of uncooked LTL muscle, Jawasreh et al. [24] underlined no significant differences between first-generation Rambouillet *callipyge* Awassi and Awassi lambs. As shown in Figure 4, the results indicate that no significant differences were observed in *CLPG* genotypes consistent with the findings of Jawasreh

et al. [24]. Previously, greater CL was reported by several authors for *CLPG* mutated lamb meat [6,10,21]. Contrary to expectations, this study finds a lower CL of LTL muscle in *CLPG* mutated lambs than normal ones.

In our study, no genotype \times time interaction on color parameters of LTL muscle was detected consistent with the data obtained by Penick et al. [6]. However, the *MN* genotype was slightly darker during the storage period and became redder and less yellow after 48th h. These results differ from well-known previous studies that *CLPG* mutated meat is more yellow, slightly less red, and lighter than normal carcasses [24,34,37]. A possible explanation for this might be that the amount of myoglobin, mitochondria, and lipid determines the lightness of a meat color in Type I muscle fiber [38].

The present study makes several noteworthy contributions to livestock science and lamb producers. One of the most notable findings reported here is that all meat-type sheep breeds, except R, were polymorphic, suggesting that heterozygous individuals who received the *CLPG* mutation from the sire should be adopted in selection programs. The other was *CLPG* mutated lambs had higher SW, CCW, and a higher percentage of the wholesale neck, shoulder, rack, and leg. Furthermore, it is necessary to clarify exactly why the indigenous K and its crossbreeds (GBK and KM) had a great potential to improve carcass weight and leaner meat. Considerably more work will need to be done to confirm the effect of *CPLG* mutation on neck percentage with a greater number of *CLPG* mutated lambs. Studies with larger genotype numbers could provide more definitive evidence about carcass characterization and quality of these meat-type breeds.

Acknowledgment and/or disclaimers

The present study was supported by the Republic of Turkey, Ministry of Agriculture and Forestry, General Directorate of Agricultural Research (Project No: TAGEM/HAYSUD/B/18/A4/P2/308).

Conflict of interest

The authors declare that they have no competing interests.

Compliance with ethical standards

The animal care and handling procedures were reviewed and approved by the Ethical Committee of the Sheep Breeding Research Institute (approval number:13360037)

References

1. Tolee Al RR., Olga E, Ekaterina C. Identification of *CLPG* gene polymorphism using PCR-RFLP of Iraq and Belarus population sheep breeds. *Gene Reports* 2021; 22: 100974. doi: 10.1016/j.genrep.2020.100974
2. Margawati ET, Raadsma HW, Martojo H, Subandriyo, Muladno. Quantitative Trait Loci (QTL) Analysis for Production Traits of Birth Weight and Weight 360 days in Backcross Sheep. *HAYATI Journal of Biosciences* 2006; 13: 31-35. doi: 10.1016/S1978-3019(16)30376-X

3. Nanekarani S, Goodarzi M. Polymorphism of Candidate Genes for Meat Production in Lori Sheep. *IERI Procedia* 2014; 8: 18-23. doi: 10.1016/j.ieri.2014.09.004
4. Cockett NE, Jackson SP, Shay TL, Nielsen D, Moore SS et al. Chromosomal localization of the callipyge gene in sheep (*Ovis aries*) using bovine DNA markers. *Proceedings of the National Academy of Sciences* 1994; 91: 3019-3023. doi: 10.1073/pnas.91.8.3019
5. Othman OE, Balabel EA, Mahfouz ER. Genetic characterization of myostatin and callipyge genes in Egyptian small ruminant breeds. *Biotechnology* 2016; 15: 44-51. doi: 10.3923/biotech.2016.44.51
6. Penick M, Kim HW, Setyabrata D, Waddell JN, Bidwell CA et al. Callipyge genotypic effects on meat quality attributes and oxidation stability of ovine *M. longissimus*. *Small Ruminant Research* 2017; 146: 5-12. doi: 10.1016/j.smallrumres.2016.11.011
7. Delgado EF, Geesink GH, Marchello JA, Goll DE, Koohmaraie M. The calpain system in three muscles of normal and callipyge sheep. *Journal of Animal Science* 2001; 79 (2): 398-412. doi: 10.2527/2001.792398x
8. Cramer T, Penick ML, Waddell JN, Bidwell C., Kim YHB. A new insight into meat toughness of callipyge lamb loins - The relevance of anti-apoptotic systems to decreased proteolysis. *Meat Science* 2018; 140: 66-71. doi: 10.1016/j.meatsci.2018.03.002
9. Ma D, Yu Q, Hedrick VE, Cooper BR, Paschoal Sobreira TJ et al. Proteomic and metabolomic profiling reveals the involvement of apoptosis in meat quality characteristics of ovine *M. longissimus* from different callipyge genotypes. *Meat Science* 2020; 166: 108140. doi: 10.1016/j.meatsci.2020.108140
10. Busboom JR, Wahl TI, Snowden GD. Economics of callipyge lamb production. *Journal of Animal Science* 1999; 77 (Suppl 2): 243-248. doi: 10.2527/1999.77suppl_2243x
11. Cockett NE, Smit M, Bidwell CA, Segers K, Hadfield TL et al. The callipyge mutation and other genes that affect muscle hypertrophy in sheep. *Genetics Selection Evolution* 2005; 37: 65-82. doi: 10.1051/gse:2004032
12. Kader Esen V, Esen S, Karadağ O, Önenç A, Elmaci C. Slaughter and carcass characteristics of Kivırcık, Karacabey Merino, Ramlıç, German Black-Head Mutton × Kivırcık and Hampshire down × Merino crossbreed lambs reared under intensive conditions. *Turkish Journal of Veterinary & Animal Sciences* 2020; 44: 1155-1163. doi: 10.3906/vet-2006-24
13. Kader Esen V, Elmaci C. 2021. The estimation of live body weight from linear body measurements in different meat-type lambs. *Journal of Agricultural Sciences* 2021; 27 (4): 469-475. doi: 10.15832/ankutbd.719037
14. Qanbari S, Osfoori R, Eskandarinasab MP. A preliminary study of marker data applicability in gene introgression program for Afshari sheep breed. *Biotechnology* 2007; 6(4): 513-519.
15. Knowles SO, Grace ND, Rounce JR, Realini CE. Quality, nutrient and sensory characteristics of aged meat from lambs supplemented with selenomethionine. *Food Research International* 2020; 137: 109655. doi: 10.1016/j.foodres.2020.109655
16. Honikel KO, Hamm R. Measurement of water-holding capacity and juiciness. In: Pearson AM, Dutson TR (editors). *Quality attributes and their measurement in meat, poultry and fish products*. Boston, USA: Springer; 1994. pp. 125-161.
17. Choi YM, Kim BC. Muscle fiber characteristics, myofibrillar protein isoforms, and meat quality. *Livestock Science* 2009; 122 (2-3): 105-118. doi: 10.1016/j.livsci.2008.08.015
18. Gonzales-Barron U, Santos-Rodrigues G, Piedra RB, Coelho-Fernandes S, Osoro K, C et al. Quality attributes of lamb meat from European breeds: Effects of intrinsic properties and storage. *Small Ruminant Research* 2021; 198: 106354. doi: 10.1016/j.smallrumres.2021.106354
19. Yeh FC, Yang RTJ, Xiyang JM. PopGene32. Microsoft window-based freeware for population genetic analysis, Version 1.32 (Software); University of Alberta: Edmonton, AB, Canada: 2000.
20. Minitab I: Statistical software for windows, release 17. Minitab Inc., USA, 2014.
21. Abdulkhaliq AM, Meyer HH, Busboom JR, Thompson JM. Growth, carcass and cooked meat characteristics of lambs sired by Dorset rams heterozygous for the Callipyge gene and Suffolk and Texel rams. *Small Ruminant Research* 2007; 71: 92-97. doi: 10.1016/j.smallrumres.2006.05.005
22. Busboom JR, Hendrix WF, Gaskins CT, Cronrath JD, Jeremiah LE et al. Cutability, fatty acid profiles and palatability of callipyge and normal lambs. *Journal of Animal Science* 1994; 72 (Suppl 1): 60.
23. Cockett NE, Jackson SAMP, Snowden GD, Carpenter CE. Characterization of the Callipyge Trait in Sheep. In: *Proceedings of the 49th Annual Reciprocal Meat Conference*; Provo, Utah, USA; 1996. pp. 102-105.
24. Jawasreh KI, Alamareen AH, Obeidat MD, Aad PY. 2019. Growth performance and meat characteristics of the first awassi-rambouillet callipyge backcross. *Animals* 2019; 9: 1-12. doi: 10.3390/ani9080517
25. Alakilli SY. Analysis of polymorphism of caplstatin and callipyge genes in Saudi sheep breeds using PCR-RFLP technique. *International Journal of Pharmaceutical Sciences Review and Research* 2015; 30: 340-344.
26. Dimitrova I, Bozhilova-Sakova M. PCR-RFLP analysis of callipyge gene (CLPG) in Karakachan sheep breed. *Bulgarian Journal of Agricultural Science* 2016; 22: 482-484.
27. Gorlov IF, Shirokova NV, Kolosov YA, Kolosov AY, Getmantseva LV et al. Polymorphism of CLPG gene in three sheep breeds grown in the steppe zone of the Russian Federation. *Journal of Advanced Veterinary and Animal Research* 2020; 7: 51-55. doi: 10.5455/JAVAR.2020.G392

28. Jackson SP, Miller MF, Green RD. Phenotypic Characterization of Rambouillet Sheep Expressing the Callipyge Gene: II. Carcass Characteristics and Retail Yield. *Journal of Animal Science* 1997; 75: 125–132. doi: 10.2527/1997.751125x
29. Shah A, Aziz A, Ghafoor A, Zahur AB, Yousaf M et al. Molecular analysis of callipyge gene mutation (C. 267A> G) in Kajli, Lohi and Thalli sheep breeds of Pakistan. *Russian Journal of Genetics* 2018; 54 (7): 848-852. doi: 10.1134/S1022795418070141
30. Jawasreh KI, Al-Amareen AH, Abdullah AY. 2016. P7000 Growth performance and meat characteristics of Awassi sheep that holds the callipyge gene. *Journal of Animal Science* 2016; 94 (Suppl 4); 175-176. doi: 10.2527/jas2016.94supplement4175b
31. Koohmaraie M, Shackelford SD, Wheeler TL, Lonergan SM, Doumit ME. A muscle hypertrophy condition in lamb (callipyge): characterization of effects on muscle growth and meat quality traits. *Journal of Animal Science* 1995; 73(12): 3596-3607. doi: 10.2527/2001.792398x
32. Goodson KJ, Miller RK, Savell JW. Carcass traits, muscle characteristics, and palatability attributes of lambs expressing the callipyge phenotype. *Meat Science* 2001; 58: 381–387. doi: 10.1016/S0309-1740(01)00038-9
33. Hopkins DL, Hegarty RS. Effect of sire type and plane of nutrition on lamb loin shear force, myofibrillar fragmentation index and calcium concentration. *Journal of Muscle Foods* 2004; 15 (2): 109-120. doi: 10.1111/j.1745-4573.2004.tb00715.x
34. Kerth CR, Jackson SP, Ramsey CB, Miller MF. Characterization and consumer acceptance of three muscles from Hampshire x Rambouillet cross sheep expressing the callipyge phenotype. *Journal of Animal Science* 2003; 81: 2213-2218. doi:10.2527/2003.8192213x
35. Carpenter CE, Rice OD, Cockett NE, Snowden GD. Histology and composition of muscles from normal and callipyge lambs. *Journal of Animal Science* 1996; 74 (2): 388-393. doi: 10.2527/1996.742388x
36. Yu H, Waddell JN, Kuang S, Tellam, RL, Cockett NE et al. Identification of genes directly responding to DLK1 signaling in Callipyge sheep. *BMC Genomics* 2018; 19:117. doi: 10.1186/s12864-018-4682-1
37. Abdulkhaliq AM, Meyer HH, Thompson JM, Holmes ZA, Forsberg NE et al. Callipyge gene effects on lamb growth, carcass traits, muscle weights and meat characteristics. *Small Ruminant Research* 2002; 45 (2): 89-93. doi: 10.1016/S0921-4488(02)00086-X
38. Choi MJ, Abduzukhurov T, Park DH, Kim EJ, Hong GP. Effects of deep freezing temperature for long-term storage on quality characteristics and freshness of lamb meat. *Korean Journal for Food Science of Animal Resources* 2018; 38 (5): 959-969. doi: 10.5851/kosfa.2018.e28