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

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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 (Kıvırcık, Karacabey Merino, Ramlıç, German Black-Head Mutton × Kıvırcık, 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'-GGAATCATCGTGTCCTGGTC-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.

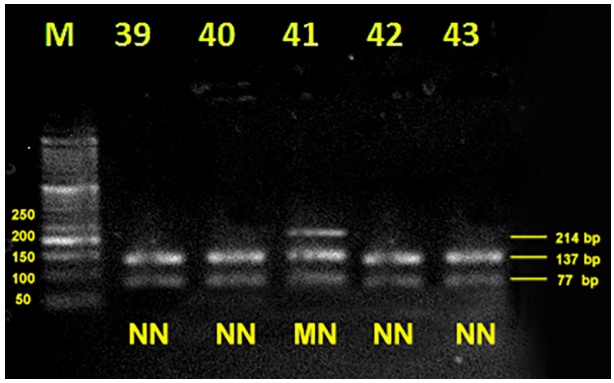

Figure 1. Band patterns of PCR-RFLP by digesting 214 bp DNA fragment of CLPG gene with the use of *AvaII* restriction endonuclease enzymes. The *MN* genotype presents three fragments (214 bp, 137 bp, 77 bp), while *NN* presents two fragments (137 bp, 77 bp).

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 SF, 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: Kıvırcık, KM: Karacabey Merino, R: Ramlıç, GBK: German Black-Head Mutton × Kıvırcık, HM: Hampshire Down × Merino.

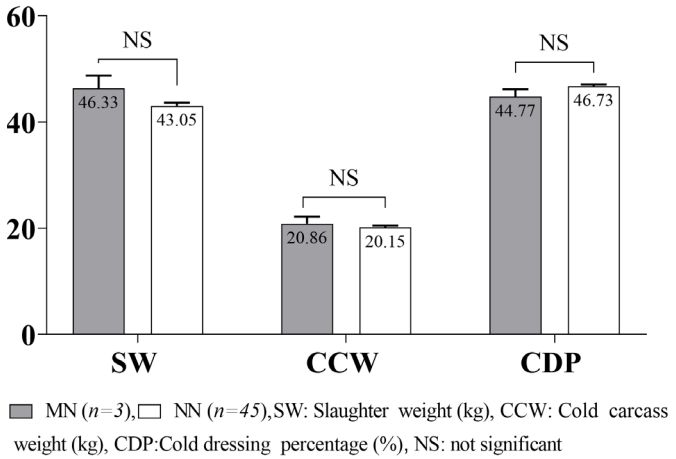

Figure 2. Slaughter weight, carcass weight, and dressing percentage of *CLPG* genotypes in meat-type lambs.

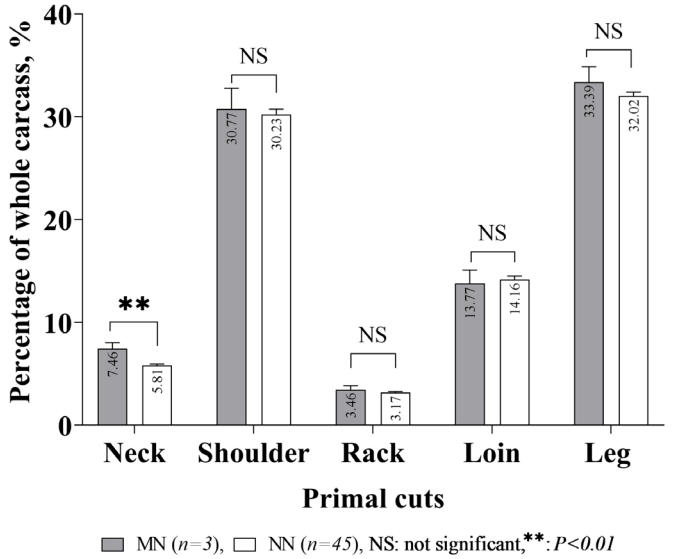

Figure 3. Retail carcass percentage of *CLPG* genotypes in meat-type lambs.

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

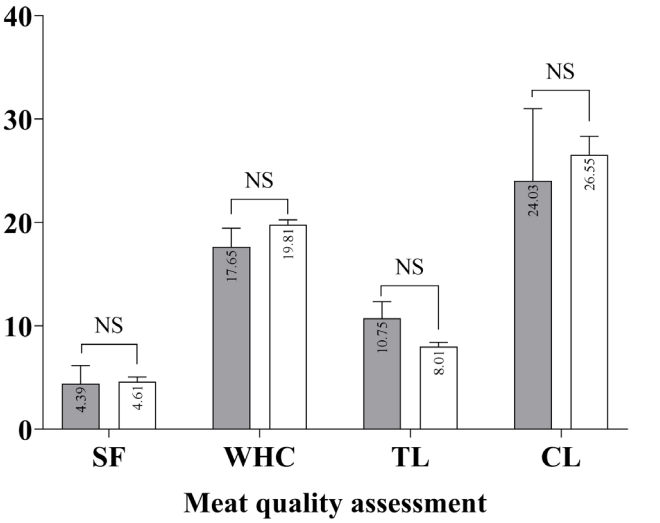

Figure 4. Meat quality assessment of *CLPG* genotypes in meat-type lambs.

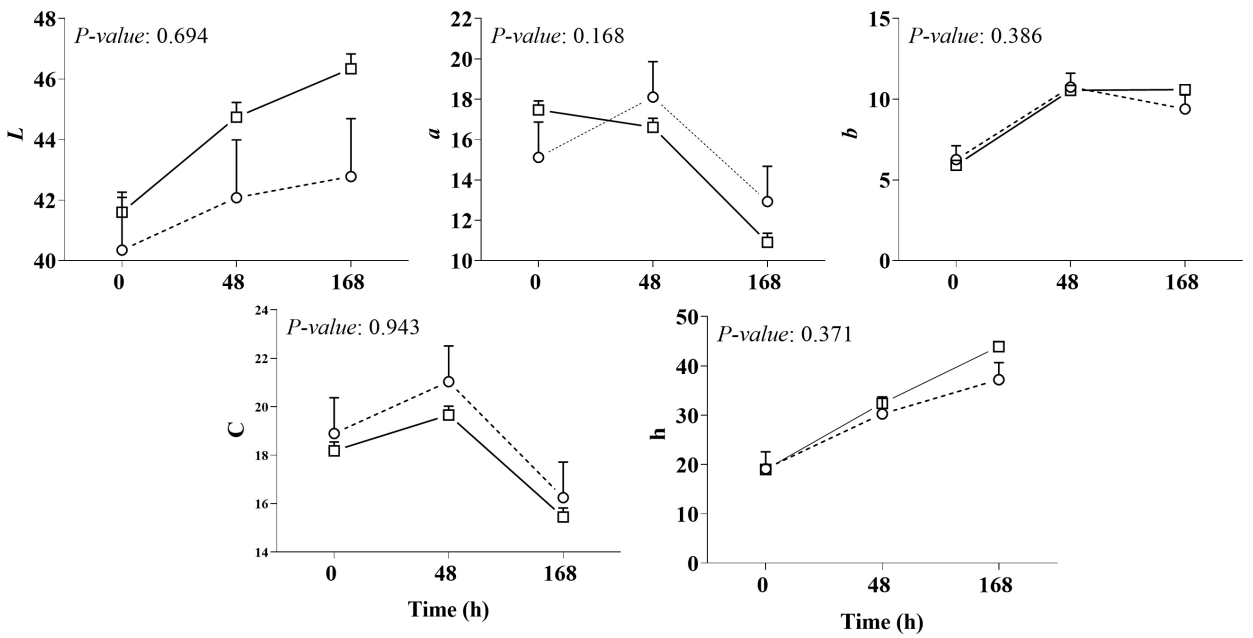

Figure 5. Genotype × time interaction on color parameters of *Longissimus thoracis et lumborum* muscle during the storage period.

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 Osssimi 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 × 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.

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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)

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