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Effect of Growth Hormone Exon-5 Polymorphism on Growth Traits, Body Measurements, Slaughter and Carcass Characteristics, and Meat Quality in Meat-Type Lambs in Turkey

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Abstract: This study examined the relationship between *GHE5* polymorphisms and growth and carcass characteristics of meat-type sheep breeds reared in Turkey. A total of 202 lambs were tested, consisting of five breeds. By SSCP analysis and nucleotide sequencing, 14 nucleotide changes (12 substitutions and two deletions) were identified in four variants of *GHE5*. In the coding region of *GHE5*, five substitutions occur, including c.1588C>Y(C/T) (Ala160Val), c.1603A>M(A/C), c.1604G>S(G/C) (Lys165Thr), c.1606A>W(A/T) (Gln166Leu), and c.1664C>Y(C/T). *P3* female and *P1* male lambs had the highest rump height at weaning, whereas *P3* females and *P2* males had the highest chest depth ($p < 0.05$). At yearling, *P1* variant lambs have longer body length (BL; $p < 0.05$), wider leg circumferences, and thinner cannon bone perimeter (CBP) ($p > 0.01$), in contrast to *P2* variant lambs, which have a shorter BL and thicker CBP. Furthermore, *P2* had a greater percentage of neck, shoulder, and leg, *P1* had a greater percentage of loin, and *P3* had a greater percentage of rack, but there was no significant difference between them. A marker-assisted selection approach can be used to improve sheep carcass quality traits by taking advantage of the nucleotide substitutions found on *GHE5* and the detected differences between variants.

Keywords: growth hormone exon-5; meat quality; carcass; slaughter; lamb; body measurements; ultrasonographic measurements; polymorphism; color

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1. Introduction

As a result of the global food shortage, researchers are striving to increase food production volumes while maintaining quality characteristics that are essential to human health. In addition to being a major source of protein, meat also contains vitamins, minerals, and fats, including fatty acids that are crucial to human nutrition [1]. It is important to develop better ways to use sheep breeds' gene pools to increase meat and milk productivity. Animal producers can improve their economic performance by reducing feed costs per unit, monitoring genetics, controlling selection, and developing supplementary reserves. By identifying genes that underlie valuable traits, undesirable traits can be eliminated, while important traits, such as litter size, growth traits, and meat quality, can remain [2,3]. Therefore, it is essential to identify the mutations that determine animals' valuable characteristics.

The use of DNA technology allows new methods for assessing animals to identify genes that are directly or indirectly related to commercially important qualities [4,5]. Moreover, it is possible to breed out the preferred gene variants directly on the DNA level and conduct genomic selections along with conventional selections.

Farm animal growth characteristics can be determined using the *GH* gene, which plays an important role in growth metabolism. *GH* regulates growth and metabolism in various

farm animals by improving body and carcass size due to its functions, such as protein synthesis and fat separation [6]. *GH* genes share 97.5% homology in sheep and cattle due to a series of five exons and four introns at 1792 base pairs (bp) on the 11th chromosome [7].

A number of farm animals, including buffalo, cows, goats, and sheep, possess the *GH* gene, and mutations in the *GH* gene affect growth, carcass weight, and milk production [8]. A change in exon-5 (*GHE5*) or intron-3 affects milk properties, while a change in the promoter region affects carcass weight and fat content [9,10]. As well, An et al. [11] demonstrated that goats can convert glycine amino acid into serine when a C>T nucleotide change occurs in exon-3 (*GHE3*), and proline can be turned into histidine when a C>A nucleotide change occurs in *GHE5*. It is important to note, however, that these changes in nucleotides do not always result in amino acid differentiation. As an example, Bahrami et al. [12] showed that, despite the presence of polymorphism between exon-4 (*GHE4*) and *GHE5*, only one (G.1507G>C) is involved in amino acid differentiation (glycine>tyrosine), despite observing two nucleotide changes (G.1486A>G, G.1489G>C) in *GHE4* and three in *GHE5* (G.1503G>C, G.1507G>C, and G.1509A>G).

In Turkey, there is a widespread use of the Merino (which reproduces throughout the year and develops earlier) and indigenous Kıvırcık (high meat quality and flavor, disease resistance, thin tail structure, and marbling) breeds, as well as crossbreeds derived from these two breeds in order to meet the demand of the market [13,14]. Furthermore, lamb meat producers, particularly in the western part of Turkey, tend to keep more than one genotype in their herd, primarily because of their curiosity about their adaptability, growth rate, and rearing or fattening practices, such as Suffolk or Ramlıç (crossbreeding Rambouillet with Daglıç) [15]. The lack of molecular characterization of these meat-type breeds prevents genetic variation from being effectively utilized in animal breeding programs. Further, to the best of our knowledge, no studies have investigated the *GHE5* polymorphism among these sheep breeds. Previous studies have also demonstrated that the SSCP method is an effective tool for determining the relationship between polymorphisms and yield traits in farm animals [16,17]. Therefore, the purpose of this study was to investigate the effects of the *GHE5* polymorphism on lamb growth traits, body measurements, slaughter characteristics, and meat quality using the SSCP method.

2. Materials and Methods

2.1. Ethical Approval

The Ethical Committee at the Sheep Breeding Research Institute in Balikesir, Turkey, cleared the investigation before commencing (Approval number: 13360037), and in the same Institute's experimental farm unit from January through June 2018, animal care and handling procedures were followed in accordance with the Declaration of Helsinki guidelines.

2.2. Animals and Feeding Regimens

In the current study, the animal background and feeding regimens were derived from the studies by Kader Esen et al. [15], Kader Esen and Elmaci [18], and Kader Esen et al. [19,20]. We studied 202 later-born lambs from five different meat-type breeds [15 Males (M) and 36 Females (F) of Kıvırcık (K), 14 M and 33 F of Karacabey Merino (KM), 14 M and 14 F of Ramlıç (R), 15 M and 34 F German Black-Head Mutton × Kıvırcık (GBK), 11 M and 16 F of Hampshire Down × Merino crossbreed (HM)] whose mean weaning age was 90.5 ± 5.7 days (mean ± SD). The lambs were housed in separate barns with their dams and suckled twice a day during the pre-weaning period. Once 15 days of age, commercial starter feed and high-quality alfalfa hay were available ad libitum. From weaning to yearling, lambs of various breeds were housed together in a single flock, and they received an average of 600 g/lamb of concentrate feed, 100 g/lamb of alfalfa hay, and 300 g/lamb of vetches–wheat mixture hay per day during the weaning period to slaughter. For the purpose of preventing unwanted matings, male and female lambs were separated after slaughter. The lambs were pastured between slaughtering and a yearling period when the

weather permitted, and they received the same amount of concentrate feed, alfalfa hay, vetches–wheat mixture hay, and wheat straw ad libitum. The chemical composition of the concentrates and roughages used in this study can be seen in Table 1.

Table 1. Chemical composition of concentrate and roughages [1].

Item	DM	CP	CA	EE	CF	NDF	ADF
Concentrate feed	89.44	12.65	9.43	3.22	9.66	39.90	12.95
Alfalfa hay	87.49	23.20	14.51	2.07	20.38	35.43	23.00
Vetch–wheat mixture hay	89.49	8.71	5.66	1.26	41.86	63.59	46.68

DM: Dry matter (g/kg fed basis); CP: Crude protein (g/kg DM); CA: Crude Ash (g/kg DM); EE: Ether extract (g/kg DM); CF: Crude fiber (g/kg DM); NDF: Neutral detergent fiber (g/kg DM); ADF: Acid detergent fiber (g/kg DM). [1] Data in the table is extracted from Kader Esen et al. [15].

2.3. DNA Extraction, Primer Design, PCR Amplification, SSCP, and DNA Sequencing

2.3.1. DNA Extraction

Before slaughtering male lambs, blood samples were collected into sterile EDTA tubes from all lambs. Genomic DNA was amplified using a GeneAll® DNA extraction kit using the Bio-Rad T100 thermal cycler.

2.3.2. Primer Design, PCR Amplification, and SSCP Analysis

PCR primers were designed by Bahrami et al. [12] for 365 bp of *GHE5*. Amplification was performed with a commercial kit GeneAll® 2XAmpMaster in a 20 µL reaction containing DNA and each primer at 100 ng. After 1 min at 95 °C, 30 cycles of 30 s at 95 °C, 30 s at 60 °C, and 30 s at 72 °C followed, with a final extension of 10 min at 72 °C are used for the exon 5 region amplified. Amplification was visualized by electrophoresis 3% agarose gels, using 1× TAE Buffer.

PCR products were mixed 1:10 with loading dye containing 98% formamide and denaturated at 95 °C for 7 min. Samples were then cooled rapidly on wet ice, and acrylamide: bisacrylamide gels (29:11) were made in accordance with Green and Sambrook [21]. A vertical electrophoresis was used for 4 h at 350 V. A silver staining technique was used, according to Byun et al. [22], by modifying the formaldehyde ratio to 2% in order to make electrophoresis bands visible.

2.3.3. DNA Sequencing

Based on the PCR-SSCP band models, DNA sequences were obtained from each pattern using an ABI PRISM genetic analyzer (Applied Biosystem, Foster City, CA, USA). We visualized chromatograms using Bioedit Sequence Alignment Editor, discarded noisy sequences, and aligned clear sequences. The NCBI GeneBank databases were searched using nucleotide differences determined by the BLAST Algorithm between the pattern and reference sequence (Accession number: AF.002110).

2.4. Live Weight, Linear Body Measurement, and In Vivo Ultrasonographic Measurements

Lambs' birth weights (BW) were recorded within 12 h of birth, while live weights (LW), linear body measurements, and ultrasonographic measurements were taken on the 90th, 180th, and 360th days of the study. Before morning feeding, lambs were weighed to avoid errors due to stomach fill. An experienced technician measured a lamb's linear body measurements while standing with its head raised. A flexible calibrated tape and calipers were used to record the body lengths (BL), withers heights (WH), back heights (BH), rump heights (RH), chest depths (CD), chest widths (CW), rump widths (RW), chest circumferences (CC), leg circumferences (LC), and cannon bone perimeters (CBP) of individual lambs [23].

With a real-time ultrasound device (Mindray DP-20) and linear veterinary ultrasound transducer (Mindray 75L50EAV) operating at 7.5 MHz, the *Musculus longissimus dorsi* depth (MLDD), fat thickness (FT), and skin thickness (ST) of lambs between the 12th and 13th

ribs were monitored after linear body measurements were recorded on each specified period [24]. Before placing the probe, the lambs were manually immobilized, their wools between the 12th and 13th ribs were parted manually, and the ultrasonic gel was used as a couplant. Measurements were taken on the left side, 4 cm away from the vertebral column. The pressure on the transducer head was kept to a minimum to prevent fat compression. The electronic calipers of the scanner were used to measure MLDD, FT, and ST after the scanned image had been captured.

2.5. Slaughter and Carcass Characteristics and Meat Quality Assessment

We randomly selected 50 male lambs from each breed in order to evaluate slaughter and carcass characteristics and meat quality. A 12-h fast and access to freshwater were provided to lambs at the Institute's slaughterhouse before slaughter, and then they were slaughtered in accordance with commercial procedures. Slaughter weight (SW) of lambs were immediately recorded before slaughter. After skinning, we removed non-carcass components (heads, skins, feet, lungs, liver, heart, spleen, testicles, and gastrointestinal tract) and recorded hot carcass weight (HCW, including kidneys and perinephric-pelvic fat). By dividing the HCW by the SW, we calculated the hot dressing percentage (HDP). Cold carcass weight (CCW) was determined after chilled carcasses were stored at −4 °C for 24 h, and cold dressing percentage (CDP) was calculated.

We harvested the *Longissimus thoracis et lumborum* (LTL) muscle between the 5th and 12th thoracic vertebrae for further analysis in the laboratory after cutting each carcass into five primal parts [neck, shoulder, rack, loin, and leg] [25]. From photographs of the chilled carcass, [area (MLDA, perimeter (MLDP), depth (MLDD), width (MLDW), and fat thickness (MLD_{FT})] and body fatness (BF) were measured using the Fiji image measurement program (Version 1.52d) [26]. In order to measure LTL color, a white tile was calibrated with a D 65 illuminant, observed at a 10° observer angle, and observed with a colorimeter (Chroma Meter CR-410; Konica Minolta, Tokyo, Japan) [27]. Averaging three measurements after storing the samples at 4 °C for 0, 48, and 168 h determined the value of lightness (L^*), redness (a^*), yellowness (b^*), chroma (C), and hue (h). The filter-paper method was used to determine water holding capacity (WHC) in accordance with Honikel and Hamm's [28] description. A sample of LTL was evaluated for thawing loss (TL) and cooking loss (CL), as previously described by Choi and Kim [29] and Gonzales-Barron et al. [30]. We measured the LTL shear force (SF) on cooked samples in the Central Research Laboratory of Namık Kemal University, Tekirdağ, Turkey. Cooked LTL samples were tested using a TA.HDplus Texture Analyser and an HDP/WBV Warner Bratzler Blade from Stable Micro Systems Ltd., Surrey, UK.

2.6. Statistical Analysis

The General Linear Model procedure of Minitab [31] was used to assess the influence of *GHE5* polymorphism on LW, linear body measurements, in vivo ultrasonographic measurements, slaughter and carcass characteristics, and meat quality. The repeat-measures analysis of variance was used to test whether there was a statistically significant difference between the color parameters. There have been previous adjustments made for the breed (K, KM, R, GBK, HM), gender (M, F), birth type (single, twin), dam age (2, 3, 4, 5, 6, 7+), genotype (*P1*, *P2*, *P3*, *P4*), time (90th, 180th, 360th days) the interaction between them.

3. Results

Eleven lambs were excluded from the study due to unclear genotyping (n = 4) and a small number of distributions (*P4*, n = 7) at the *GHE5* locus.

Using the PCR-SSCP method, four different patterns were found on PCR products on polyacrylamide gels. In the NCBI GeneBank OP535358-OP535361, four unique PCR-SSCP patterns were deposited as sequences *P1*, *P2*, *P3*, and *P4*. The sequences obtained from sanger sequencing were accelerated by the Clustal W algorithm in the Bioedit program and crosschecked with the accession number AF002110.1 (Figure 1).

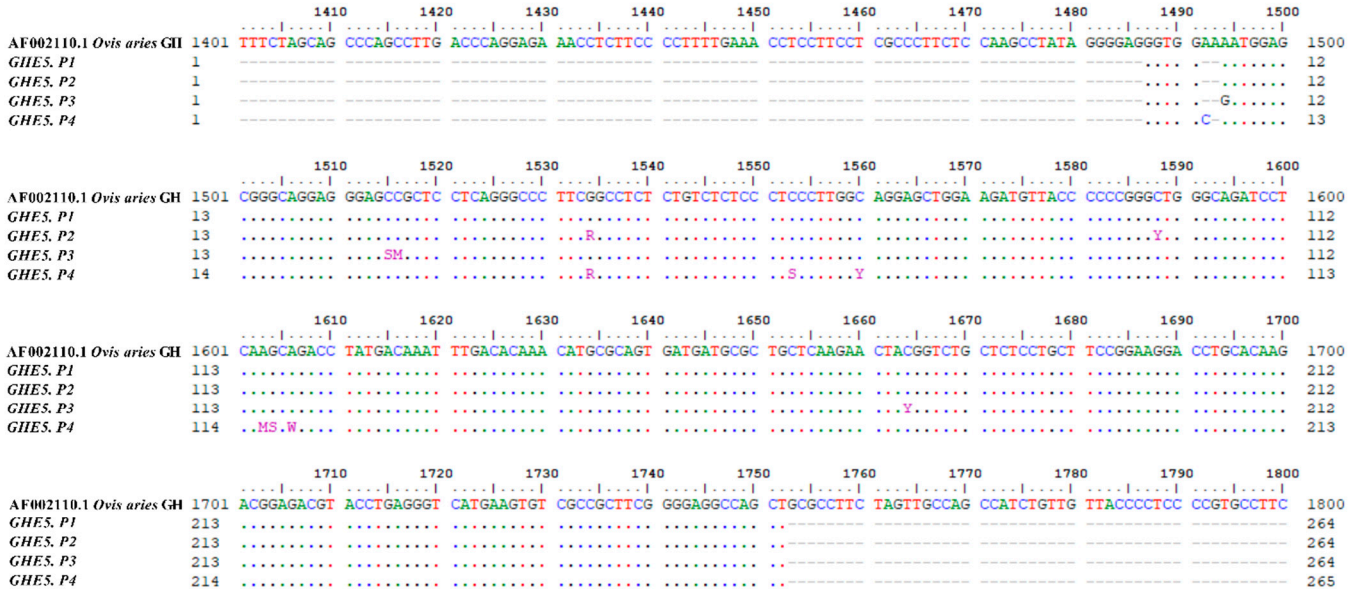

Figure 1. ClustalW algorithm-based alignment of the *GHE5* variants' sequences with the NCBI reference sequence *AF002110.1*.

Two deletions (c.1563-71_c.1563-70delAA) were detected when comparing the pattern sequences and reference sequences on *P1*, *P2*, and *P3* variants (Table 2). Moreover, two heterozygous substitutions [c.1563-29G>R(A/G) and c.1588C>Y(C/T)] were detected in the *P2* variant. *P3* variant, on the other hand, has the same deletion (c.1563-71_c.1563-70delAA) plus four substitutions [homozygous c.1563-70A>G, heterozygous c.1563-48C>S(G/C), c.1563-47C>M(A/C), and c.1664C>Y(C/T)]. There was also one deletion (c.1563-70delA) and seven substitutions [homozygous c.1563-71A>C, heterozygous c.1563-29G>R(A/G), c.1563-10C>S(G/C), C.1563-3C>Y(C/T), c.1603A>M(A/C), c.1604G>S(G/C), c.1606A>W(A/T)] were determined in *P4* variant. Further, the sequenced region contains nucleotides 1561–1763 of the encoded region, with remaining nucleotides in the non-coding region.

Table 2. Sequence variation in *GHE5*.

No	Position [1]	Nucleotide Sequences				Chromosome Location [2]	SNP rs ID	Amino Acid Changing
		P1	P2	P3	P4			
1	c.1563-71	-	-	-	C	11:14,855,698	-	-
2	c.1563-70	-	-	-	-	11:14,855,699	-	-
3	c.1563-69	A	A	G	A	11:14,855,701	-	-
4	c.1563-48	C	C	S(G/C)	C	11:14,855,722	-	-
5	c.1563-47	C	C	M(A/C)	C	11:14,855,723	-	-
6	c.1563-29	G	R(A/G)	G	R(A/G)	11:14,855,741	-	-
7	c.1563-10	C	C	C	S(G/C)	11:14,855,760	-	-
8	c.1563-3	C	C	C	Y(C/T)	11:14,855,767	-	-
9	c.1588	C	Y(C/T)	C	C	11:14,855,795	-	*p.Ala160Val*
10	c.1603	A	A	A	M(A/C)	11:14,855,809	-	*p.Lys165Thr*
11	c.1604	G	G	G	S(G/C)	11:14,855,810	-	*p.Lys165Thr*
12	c.1606	A	A	A	W(A/T)	11:14,855,812	-	*p.Gln166Leu*
13	c.1664	C	C	Y(C/T)	C	11:14,855,871	rs.596456087	-

[1] Positions are numbered according to the guidelines presented on AF002110.1. [2] Chromosome locations are given according to the guidelines presented on ENSOARG00020016511.

Further analysis of the allelic variants of the *GHE5* gene indicated no significant differences between male and female lambs in BW or LW recorded at different stages ($p > 0.05$; Figure 2). It has been observed that *P3* variants in female lambs are 2.14–10.16% heavier than other variants, whereas *P2* variants in male lambs are 2.86–8.33% heavier

than other variants. Weight differences between male and female variants of LW_{180d} (5.05 kg and 0.69 kg) began to widen over time, and this difference increased even more in adulthood (LW_{360d}; and 42.46 kg 1.34 kg). In Figure 2, it is evident that this difference is not a consequence of genotype or gender but rather a result of the breed.

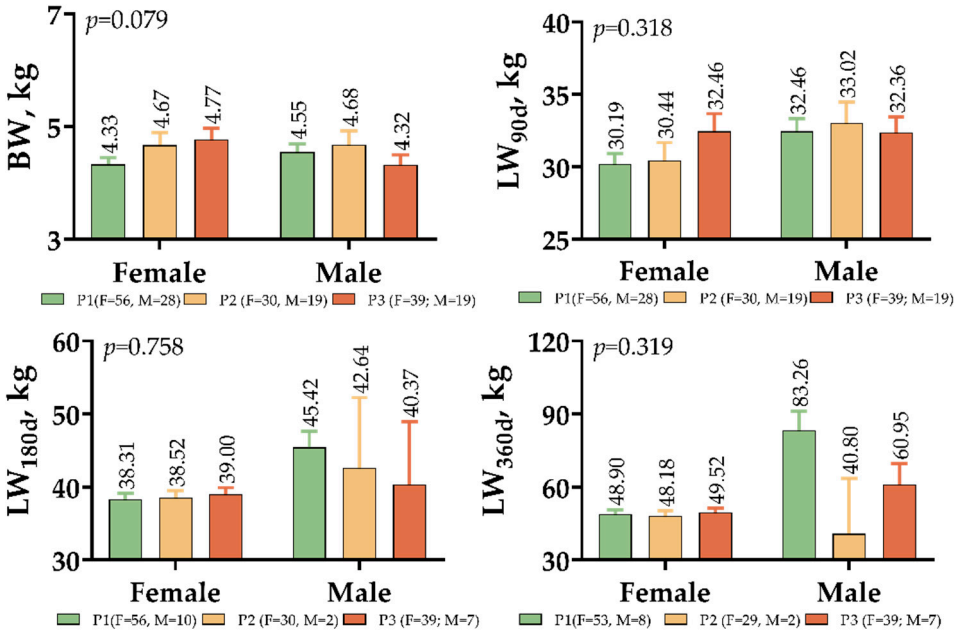

Figure 2. Effects of *GHE5* polymorphism on live weight at different periods. BW: Birth weight, LW: Live weight.

Figure S1 illustrates the effects of *GHE5* polymorphism on body measurements at different times in meat-type lambs. Statistically significant differences were seen in RH and CD between the *GHE5* variants at weaning (LW_{90d}; $p < 0.05$); and BL ($p < 0.05$), LC, and CBP ($p < 0.01$) at yearling (LW_{360d}). Nevertheless, none of the other body measurements was significantly affected at six months (LW_{180d}; $p > 0.05$). *P3* females and *P1* male lambs had the highest RH at weaning (59.68 and 59.32 cm, respectively), whereas *P3* females and *P2* males had the highest CD (24.29 and 24.60 cm, respectively) (Figure S1a). However, the difference in RH and CD disappeared at six months (Figure S1b). Although BL, LC, and CBP were similar between variants in female lambs, they differed significantly in male lambs ($p < 0.05$ for BL and $p < 0.01$ for LC and CBP; Figure S1c). At yearling, *P1* variant lambs have a longer BL, wider LC, and thinner CBP, in contrast to *P2* variant lambs, which have a shorter BL and thicker CBP.

In real-time ultrasound measurements, none of the three parameters (MLDD, FT, and ST) differed significantly between female and male lambs on the basis of *GHE5* variants ($p > 0.05$; Figure 3). Taking into account the number of *P2* variants at the sixth month and yearling, it is obvious that the MLDD of *P3* variant male lambs is higher than that of other variants.

A comparison of the carcass parameters of the *GHE5* variants is provided in Figure 4. There was no significant difference in all studied parameters ($p > 0.05$) between variants. *P1* variants recorded the highest SW (43.40 kg), HCW (20.82 kg), CCW (20.16 kg), HDP (47.94%), and CDP (46.41%), while *P2* variants recorded the highest T_{45m} (36.53 °C), T_{24h} (7.03 °C), pH_{45} (6.41), and pH_{24h} (5.56). *GHE5* variants had CLs ranging from 1.30–1.53%. Similarly, none of the *Musculus longissimus dorsi* parameters (MLDA, MLDP, MLDD, MLDW, MLD_{FT}) and BF were significant following image processing. *P2* variants had a higher value for MLDW (6.91 cm), while *P1* variants had higher values for MLDA (17.35 cm^2), MLDP (17.80 cm), and MLDD (3.42 cm). In addition, the *P3* variant showed a leaner profile than the other variants, with a lower MLD_{FT} of 8.61–9.87% as well as a lower BF of 12.86–17.86%.

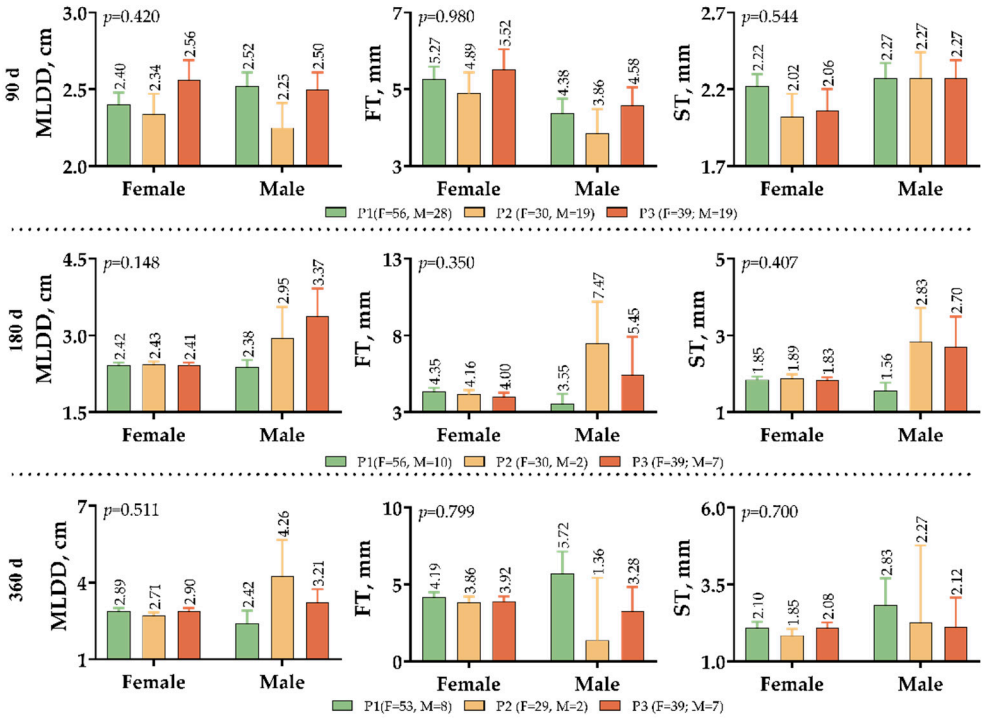

Figure 3. Effect of *GHE5* polymorphism on *Musculus longissimus dorsi* development at different periods in meat-type lambs. MLDD: *Musculus longissimus dorsi* depth; FT: Fat thickness; ST: Skin thickness.

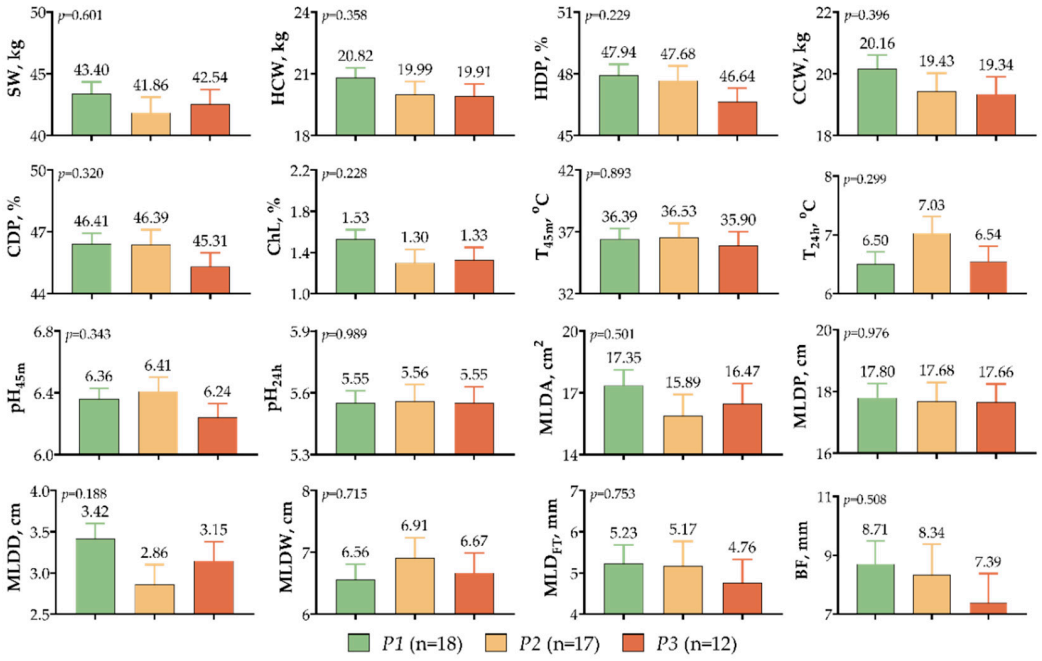

Figure 4. Effect of *GHE5* polymorphism on carcass parameters in meat-type lambs. SW: Slaughter weight; HCW: Hot carcass weight; HDP: Hot dressing percentage; CCW: Cold carcass weight; CDP: Cold dressing percentage; ChL: Chilling loss; T_{45m}: Carcass temperature after 45 min, T_{24h}: Carcass temperature after 24 h, MLDA: *Musculus longissimus dorsi* area, MLDP: *Musculus longissimus dorsi* perimeter, MLDD: *Musculus longissimus dorsi* depth, MLDW: *Musculus longissimus dorsi* width, MLD_{FT}: Fat thickness of *Musculus longissimus dorsi*, BF: Body fatness.

In Figure 5, the effects of *GHE5* polymorphism on noncarcass components are presented. There was no significant difference between the non-carcass components ($p > 0.05$). It is noteworthy that the head (0.03–0.06 kg), feet (20–60 g), testicles (41.5–72.1 g), heart (14.4–43.3 g), liver (15.0–45.7 g), spleen (18.0–36.6 g), full stomach (0.43–0.65 kg), and empty stomach (0.07–0.18 kg) weights were higher in the *P3* variant; lung (57.5–61.2 g), full intestine (0.06–0.22 kg), empty intestine (0.05–0.014 kg), omental and mesenteric fat (44.0–68.9 g), and kidney fat (5.2–11.0 g) weights were higher in the *P1* variant; skin (0.19–0.21 kg), kidney (1.5–1.9 g), and other (red offals) weights (33.8–58.6 g) in the *P2* variant.

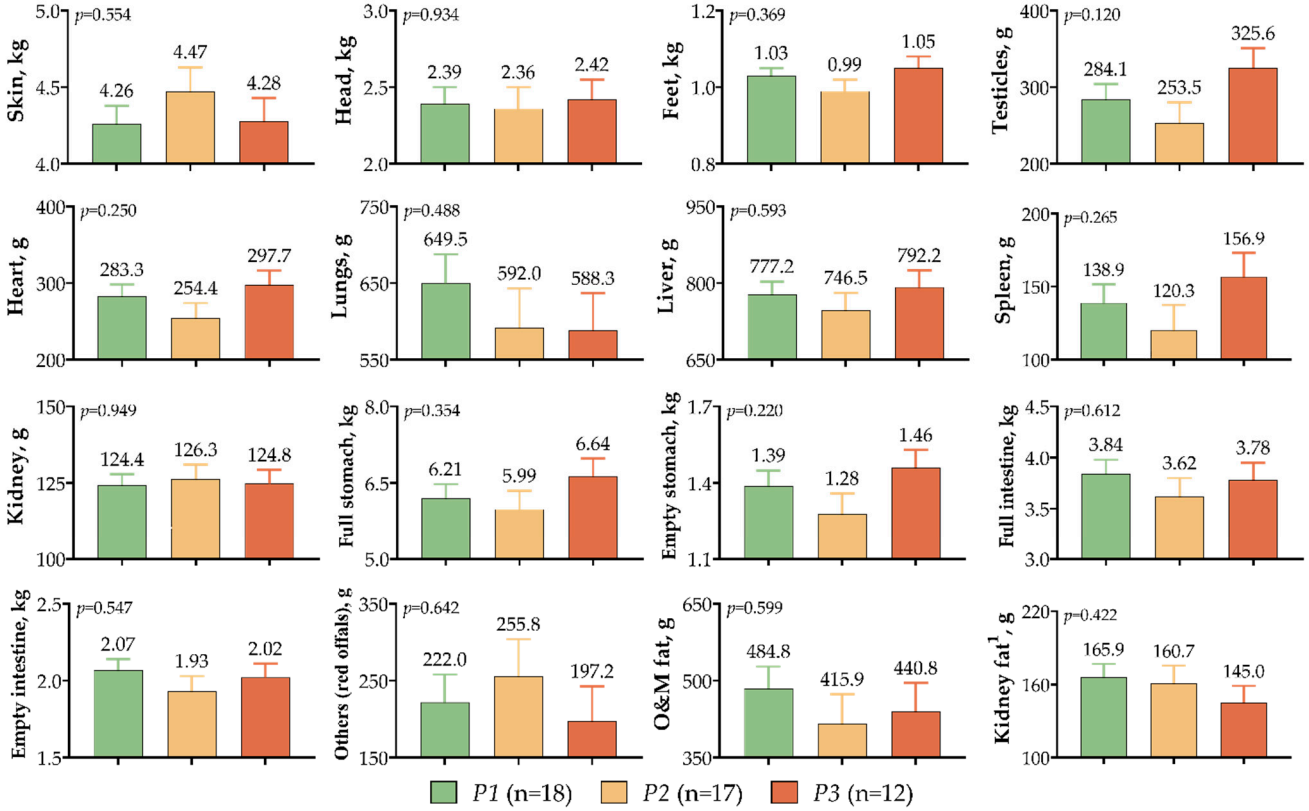

Figure 5. Effect of *GHE5* polymorphism on non-carcass components in meat-type lambs. O and M fat: Omental and mesenteric fat. [1] weight after chilling at 4 °C for 24 h.

The *GHE5* polymorphism had no significant effect on meat-type lamb neck, shoulder, rack, loin, or leg proportions, as shown in Figure 6. There was a higher neck (0.14–0.30%), shoulder (0.18–0.74%), and leg (0.08–0.42%) percentage in the *P2* variant, whereas the *P3* variant had a higher rack (0.71–1.11%) percentage, and the *P1* variant had a higher loin (0.46–0.70%) percentage.

A comparison of the meat quality assessment of *GHE5* variants in meat-type lambs is shown in Figure 7, showing the highest SF (5.05 kg), WHC (20.15%), and TL (8.65%) observed in the *P2* variant and the highest CL (28.88%) in *P3* variant. Nevertheless, none of these values were statistically significant ($p > 0.05$).

As shown in Figure 8, no significant interactions between genotype and time were observed for all color parameters at various storage periods ($p > 0.05$). A clear trend can be observed in terms of L^* and h values in all variants. After 48 h of storage, a decrease in a^* and C was detected up to the 168th h. At the end of the storage period, the *P2* variant had higher L^*, b^*, and h values than the other variants but had lower a^* and C values.

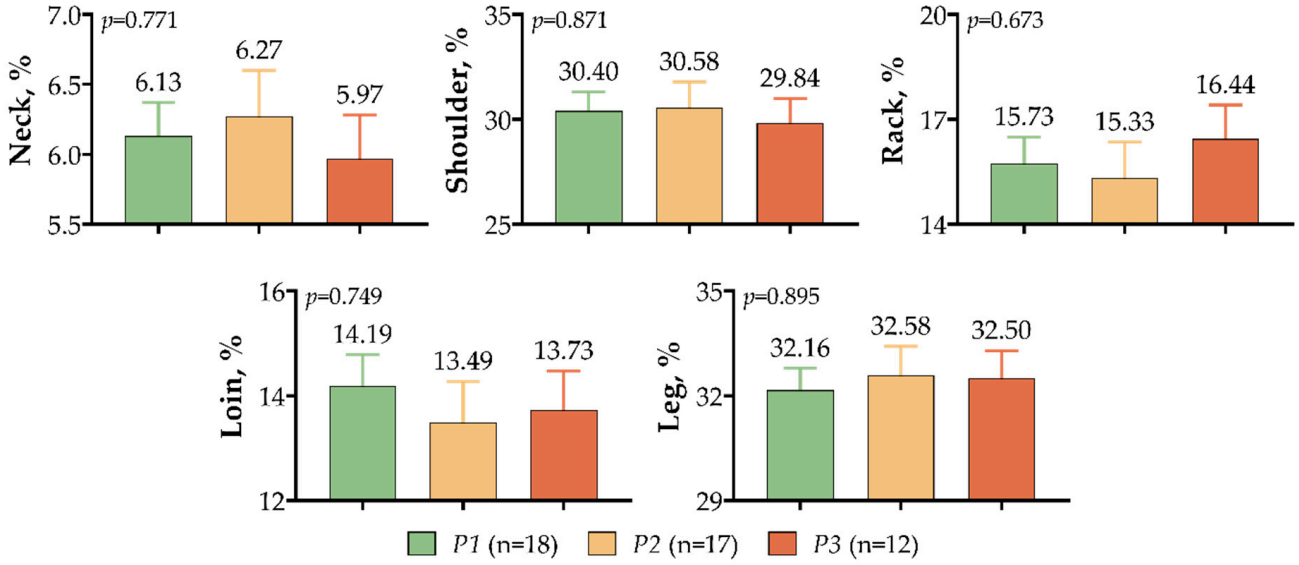

Figure 6. Effect of *GHE5* polymorphism on retail carcass percentage of meat-type lambs.

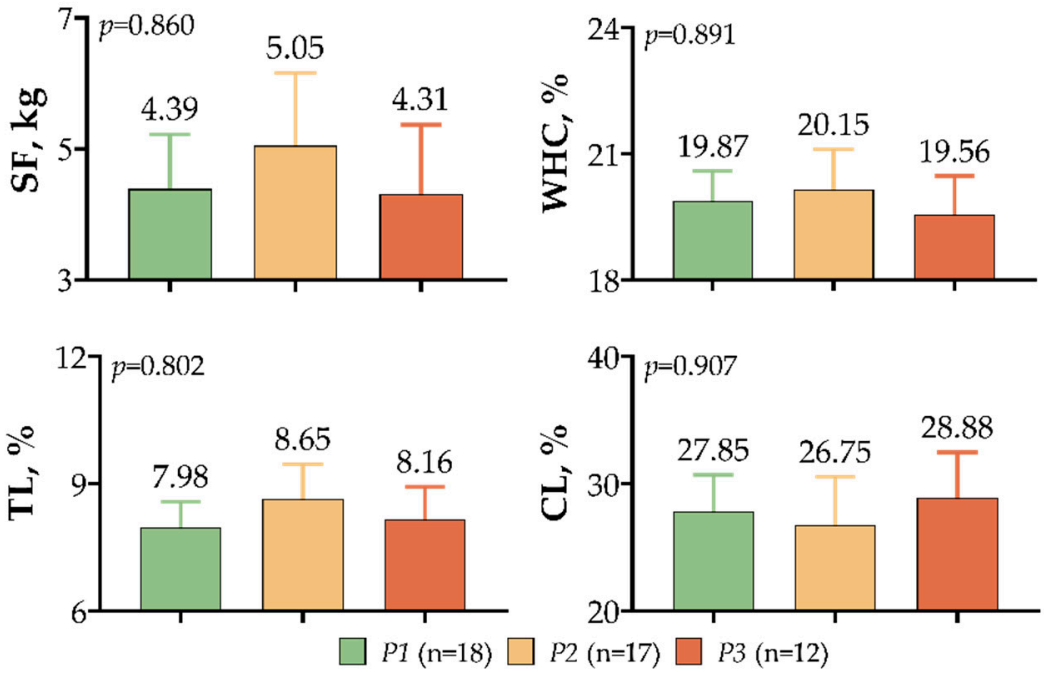

Figure 7. Effect of *GHE5* polymorphism on meat quality assessment SF: Shear force; WHC: Water holding capacity; TL: Thawing loss; CL: Cooking loss.

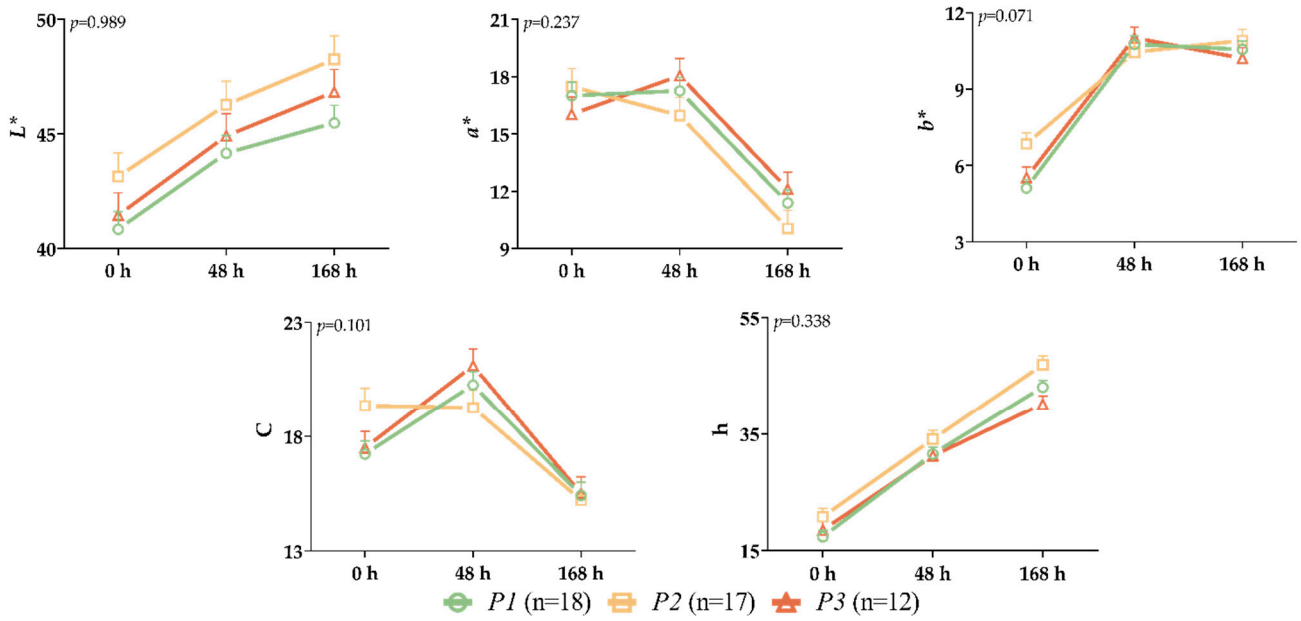

Figure 8. Genotype × time interaction on color parameters of *Longissimus thoracis et lumborum* muscle during the storage period. *L**: lightness, *a**: redness, *b**: yellowness, C: chroma, h: hue.

4. Discussion

A major objective of genomic selection is to select animals with the most superior performance and to transmit them to the next generation [32]. If structural and functional genomic approaches are combined, phenotypic differences between animals can be studied from a completely different perspective, which may lead to differences in final products [33]. Therefore, identifying genetic markers and candidate genes associated with economically important traits is highly valuable from both a biological and practical perspective [34].

In several studies, the *GH* gene, which is polymorphic in farm animals, has been shown to play a key role in growth, development, carcass quality, and milk yield [6,8,10,35]. Based on polyacrylamide gel electrophoresis bands determined by the SSCP method, our study confirms that *GH* levels can influence growth changes in meat-type lambs. Similar results were reported by Bastos et al. [36] in Churra de Quente sheep, where they detected two variants of *GHE4* and five variants of *GHE5*. However, some research indicates that different exons of *GH* are monomorphic in different breeds [37].

In a study conducted to determine *GH* polymorphism in the Salsk sheep breed by using the PCR-RFLP method and endonuclease *HaeIII* restriction enzyme, Gorlov et al. [38] found three different genotypes (*AA*, *AB*, and *BB*). They also revealed that weaning weight, weight at the age of nine months, and the average daily gain of the ram lamb with the *AB* genotype exceeded the values of these parameters of the ram lamb with the *AA* genotype by 0.92 kg, 10.67 kg, and 47.3 g, respectively. The same method and enzyme were applied in another study using Boer goat bucks, and two SNPs were identified by *GH* gene sequencing [A781G (Ser/Gly35) and A1575G (Leu147)]. Researchers found that *CC* genotypes were taller than *CD* genotypes and that Boer bucks with *AA* genotypes were born with smaller CCs at birth [39]. This study, however, detected four different variants using the PCR-SSCP method, of which one (*P4*) was excluded from statistical analysis. In the study, we found that females carrying the *P3* variant had weights of 100–440 g at birth, and males carrying the *P2* variant weighed 130–360 g more than those carrying the other variants. At other periods, the *P3* variant did not show increased values for females; however, after the sixth month, the *P1* variant displayed higher values for males (Figure 2).

It has been shown that c.1286T>C nucleotide changes affect lamb LW, WH, BL, and CW in Tibetan and Poll Dorset sheep [40]. Similarly, five variants of *GHE4* affect Makooei sheep's LW and weight at six months and nine months [41]. The SNPs G871A, G1383A, and A1509G affected Harri sheep's LW [42]. Awassi ewes' *GH* gene variant affects lamb BW as well as the WH, RH, and CC between birth and weaning [43]. Furthermore, an SNP on the *GH* gene intron 2 region (C>A) significantly affects the LW of Santa Ines sheep on the 100th day [44]. *GHE5* polymorphism significantly affects RH and CD at weaning (90th day) (Figure S1a). This can be attributed to c.1563-48>S(G/C), c.1563-47>M(A/C), c.1664>Y(C/T) nucleotide substitutions. Therefore, we thought that causes the higher RH and wider CD in female lambs with the *P3* variant, but this effect dissipated as time progressed. During the weaning period, the highest levels of RH and CD were observed in males of *P1* and *P2* variants, respectively. It is possible that the c.1588>Y(C/T) mutation resulting in the change of amino acids (p.Ala160Val) may lead to a wider CD in male lambs with the *P2* variant, whereas wild-type *P1* males have a higher RH. Furthermore, there was no significant difference between wild-type and mutant female *GHE5* variants, but wild-type male *P1* variants had a higher BL and LC, and *P3* variants had a thinner CBP than other male *GHE5* variants at yearling (Figure S1c). This can be attributed to c.1603A>M(A/C), c.1604G>S(G/C), c.1606A>W(A/T) nucleotide substitutions, which are leading to aminoacid changes (p.Lys165Thr, p.Gln166Leu). Therefore, we thought that causes the CBP to be thicker in male lambs with the *P3* variant.

Considering that 97.5% homology exists between the bovine and sheep *GH* genes, it may make sense to evaluate such studies together since studies on polymorphism, and meat quality in sheep are limited in the literature. Previous studies have demonstrated that *GH* gene variants affect the MLDA of Hereford and Limousin cattle as well as the 559G>A SNP in Hanwoo cattle [45,46]. It was found that the *GHE5* polymorphism did not have a significant effect on *Musculus longissimus dorsi* development over time ($p > 0.05$) (Figure 3). Despite the fact that our results differ from Lee et al. [45] and Sedykh et al. [46], they were similar to Özay's [47] study, which demonstrated that *GHE1* polymorphism did not affect the measurement of MLD in Kıvırcık sheep.

A close relationship has been shown between *GH* and *IGF1* since *GH* stimulates the liver to express *IGF1*, which negatively affects pituitary *GH* production [48,49]. Thus, it is possible to select carcass traits correlated with body growth by considering polymorphisms in *GH* and *IGF1* in this case [49]. The carcass parameters did not differ significantly between *GHE5* variants ($p > 0.05$). However, the SW, HCW, and CCW of *P1* variants were higher than those of the other variants by 0.86–1.54 kg, by 0.83–0.91 kg, and by 0.73–0.82 kg, accordingly (Figure 4). In addition, *P1* variants had higher MLDA (17.35 cm^2), MLDP (17.80 cm), and MLDD (3.42 cm) values, whereas *P3* variants had lower BF (7.39 mm) and MLD_{FT} (4.76 mm).

It is rare to find studies exploring association tests with sheep *GH* polymorphisms. Salsk sheep were first associated with *GH/Hae*III polymorphisms in a study by Gorlov et al. [38] with carcass traits such as carcass weight and yields, as well as heart and kidney weights. Meira et al. [49] then found that an SNP (rs589527314) was associated with carcass weights and yields as well as carcass finishing scores in Santa Ines sheep. In contrast to Gorlov et al. [38] and Meira et al. [49], this study did not demonstrate any effect of the *GHE5* polymorphism on non-carcass components ($p > 0.05$; Figure 5). On the other hand, cattle *GH* polymorphisms have been researched more extensively. In accordance with our results, there has been no significant effect of GH polymorphism on carcass yields in Indonesian domestic cattle breeds [50] or *GHE5* polymorphism in Zavot cattle [51]. Akçay et al. [51] also found that *GHE5* polymorphism does not affect either LW nor CW in Zavot cattle. Furthermore, Han et al. [52] found that Hanwoo cattle with the *GH1 Leu/Leu* genotype have higher SW, CW, BF, MLDA, and marbling scores, but no statistical significance was found. In addition, it was found that the *bGH* genotype had a significant effect on yearling weight in Canchim cattle, with positive effects associated with the *LV* (*leucine/valine*) genotype [53]. As compared to homozygous Brangus bulls exhibiting

the *GH-Msp*I RFLP genotype, Hua et al. [39] indicated that heterozygous bulls with the genotype showed higher carcass ultrasound measurements and average daily gain.

There was no significant difference in SF between *CC*, *CD*, and *DD* genotypes of bovine *GH*, as found by Costello et al. [54]. Likewise, Han et al. [52] demonstrated that *GH1* polymorphism did not significantly affect the meat and fat color, as defined by Korean legal grading standards where carcass and fat are scored between 1 and 7 in Hanwoo cattle. Similar to these studies [52,54], *GHE5* polymorphisms did not affect meat color, a key criterion for determining meat quality.

5. Conclusions

In the current study, we investigated the association between *GHE5* polymorphisms and meat quality characteristics in meat-type sheep breeds reared in Turkey using PCR-SSCP. The relation between *GHE5* variants and several growth and carcass traits, including body measurements, MLD development, weights, carcass, and noncarcass components, retail carcass percentage, meat quality, and color change during storage, was established. In conclusion, through the use of nucleotide substitutions on *GHE5* and detected differences between variants, a marker-assisted selection approach can improve sheep carcass quality traits. It is also recommended that the results obtained be confirmed with studies conducted on a larger population.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ruminants2040029/s1. Figure S1: Effect of *GHE5* polymorphism on body measurements at different periods in meat-type lambs.

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