JRVM
Journal of Research in Veterinary Medicine

RESEARCH ARTICLE

J Res Vet Med. 2025: 44 (2) 133-145
DOI:10.30782/jrvm.1798308

Epistatic Interaction Between *LEP* and *MYF5* is Associated with Carcass Traits in Holstein–Friesian Bulls

Nursen SENTURK[1]*, Tugce Necla SELVI[2], Huseyin BABAYEV[3], Hakan USTUNER[2], Sena ARDICLI[1,3]

1Bursa Uludag University, Faculty of Veterinary Medicine, Department of Genetics, Bursa, Türkiye.
2Bursa Uludag University, Faculty of Veterinary Medicine, Department of Animal Science, Bursa, Türkiye.
3University of Zurich, Swiss Institute of Allergy and Asthma Research, Davos, Switzerland.

Received 06-10-2025 Accepted 11-12-2025

Abstract

This study aims to investigate the relationship between carcass and meat quality traits and Insulin-like growth factor 1 (*IGF1*), myogenic factor 5 (*MYF5*) and *lep*tin (*LEP*) genes in Holstein-Friesian bulls. One hundred three cattle were genotyped using the PCR-RFLP method. Afterwards, the relationship of *IGF1*, *MYF5* and *LEP* genes with live weight, hot carcass weight, chilled carcass weight, dressing percentage, musculus longissimus dorsi, Warner-Bratzler shear force, cooking loss, chilling loss, water holding capacity, carcass length, chest width, and meat color parameters (*L*, a*, b**) were examined. Trade-offs between carcass and meat quality were assessed using a Pareto-front approach, where non-dominated genotype combinations were identified as the best attainable balance between yield and quality. No significant association was observed between *IGF1*, *MYF5*, and *LEP* genes individually and traits (P>0.05). However, *LEP* × *MYF5* interaction was observed in association with live weight, hot carcass weight, and chilled carcass weight traits (P<0.05). This genotypic interaction revealed an epistatic pattern that may significantly affect the live weight, hot carcass weight, and chilled carcass weight of Holstein-Friesian cattle. To our knowledge, although the individual associations of *LEP* and *MYF5* with carcass traits in Holstein–Friesian cattle have been reported, the joint (epistatic) effect of these loci has not been systematically evaluated. The present study provides the first evidence that the *LEP* × *MYF5* interaction is associated with carcass performance, whereas no corresponding interaction was detected for standard meat-quality endpoints. These findings support incorporating epistatic marker combinations, rather than single-locus effects alone, into selection indices and mating plans to improve carcass yield. More broadly, the results motivate validation in independent populations and further investigation of gene–gene and gene–environment interactions underlying carcass and meat-quality traits.

Keywords: Beef production; gene interaction; cattle; SNP

Introduction

Carcass and meat quality in cattle are crucial for achieving a products that meets consumer demands. Carcass traits are assessed using numerous phenotypic criteria, including carcass weight, muscle length, marbling, fat thickness, meat color, and pH, with varying levels of heritability (1). Modern genetic approaches enable the identification of specific genes influencing carcass quality and support their effective utilization in breeding programs (2, 3). Carcass quality is not only an economic factor but also a performance parameter that can be directly targeted for genetic improvement (4). Studies conducted within this framework increasingly emphasize the importance of genetically based strategies in cattle breeding. Genes regulating muscle development, fat metabolism, and growth factors are critical to understanding the genetic basis of carcass quality. In this context, the *lep*tin (*LEP*) gene, the insulin-like growth factor 1 (*IGF*1) gene, and the myogenic factor 5 (*MYF5*) gene are prime candidates for understanding carcass prop-

* Corresponding author: Nursen SENTURK, MSc, entrknursen@gmail.com, Tel: (+90) 224 294 1307

erties in cattle.

Genetic variations in the *LEP* gene, which plays an important role in regulating and controlling the productive performance of animals, have been reported to be associated with energy balance, milk production, body weight and fertility traits in various animal species (5- 8). Studies in the literature examine the relationship between *LEP* and *LEPR* gene polymorphisms and productivity traits such as milk yield and milk composition, carcass and meat quality traits, and morphological characteristics in cattle. (9-14). Furthermore, significant associations have been observed with productivity and reproductive traits in goats (15-17). The *LEP* is located on chromosome 4 in the cattle genome and consists of three exons and two introns (18). A SNP in exon 3 of the cattle *LEP* gene codes for alanine instead of valine at position 80 (19, 20). This polymorphism has been reported in the literature as a *Candida*te gene for carcass and meat quality traits (21, 22). The significance of *LEP* in cattle production is further highlighted by its pleiotropic effects, which have been linked to immunological function, growth rate, and meat quality (23). *IGF*1, an important *Candida*te gene for regulating musculoskeletal development, has been associated with yield traits such as carcass muscle mass, hot carcass weight, and muscle area by affecting the proliferation and differentiation of muscle cells (24). *IGF*1 is located on bovine chromosome 5 and has six exons (25, 26). The thymine/cytosine transition (T/C) at position 472 of the 5'-non-coding region of the *IGF*1 gene has been reported as a *Candida*te marker for growth performance and carcass traits (27-29). Specific polymorphisms in the *IGF*1 gene have been shown to have significant associations with meat quality traits, including external fatness and fat loss, as well as color parameters such as brightness and color values in beef (30). These associations suggest that variations in *IGF*1 may significantly affect both the physical properties and overall meat quality. The *MYF5* gene is one of the myogenic regulatory factors in the muscle regulatory factors (MRF) gene family (31). It regulates the proliferation of myoblasts by activating muscle progenitor cells (32, 33). In this respect, it is the muscle-specific factor associated with the myoblastic lineage. It is located on chromosome 5 and consists of 3 exons. It has been determined in the literature that the relationship between the g.1911A>G polymorphism located in the second intron of *MYF5* and carcass quality in different cattle breeds was examined. For example, a significant relationship has been reported between the muscle longissimus dorsi area, muscle density and carcass muscle ratio (34). The *MYF5* gene, which is known to play a role in muscle differentiation and development, also reveals that its expression levels are not only a developmental factor but also impact productive efficiency in some studies (35). The effect of *MYF5* on muscle fiber composition becomes another critical target for genetic evaluation in beef production, as it can directly impact meat tenderness and overall quality.

LEP, *IGF*1 and *MYF5* genes are *Candida*te genes that play important roles in influencing various phenotypic traits such as growth, muscle development, fat accumulation, and meat color, which are necessary to meet consumer demands and optimize agricultural practices. Increasing the market value of meat from breeds preferred for higher milk yield, such as the Holstein-Friesian breed, can increase economic value and allow for higher yield. Dairy breeds such as Holstein-Friesian are generally not well suited for fattening, and in many countries, beef production is carried out almost exclusively with beef-specific breeds. However, in countries like Türkiye, where there is a deficit in meat production, males from dairy cattle occupy a significant place in the market. An additional advantage is that many genes associated with muscle metabolism are also related to milk yield. Therefore, adopting an integrative approach to these traits may be a rational strategy, particularly in situations where meat demand cannot be balanced with production. For this reason, the genetic evaluation of Holstein males is not entirely impractical. Importantly, this approach should not be considered as a genomic selection model alone, but rather as the identification of effective gene combinations, with a stepwise analysis that also takes into account milk production characteristics. Taken altogether, this study aims to determine the relationship between *LEP*, *IGF*1 and *MYF5* genes and their interactions with carcass and meat quality traits in Holstein-Friesian cattle.

Materials and Methods

Animal Sampling

A total of 103 purebred Holstein-Friesian cattle were used in the study. All animals belonged to the Pedigree Project of the Turkish Ministry of Agriculture and Forestry and the Cattle Breeders Association. Semi-open stall settings were used for the investigation. The animals were housed in pens with concrete floors and provided with *ad libitum* food. After 15 days of training, the fattening phase began. The cattle were fed organic silage and barley straw as roughage, along with barley flour, pasta, corn, corn gluten meal, corn bran, soybean meal, and a feed additive premix. Water was available to the animals at all times during the experiment. During the fattening period, the amount of concentrate and roughages in the feed was changed in proportion to the animals' increased live weight (LW). All

procedures were conducted in compliance with national regulations.

Carcass characteristics and meat quality analysis

After the removal of non-carcass components, the hot carcass weight (HCW) was obtained. By HCW, the dressing percentage (DP) was computed, chilled carcass weight (CCW) and chilling loss (CHL) were determined after 24 hours at 4°C. Following slaughter, measures of the carcass, such as its length (CL) and chest width (CW), were taken in accordance with the anatomical locations listed by Sagsoz et al. (36). In this contex, CL is the distance between the os pubis and the first rib's tip; CW is the distance between the sixth rib's tip and the sixth vertebra on the outside of the half carcass section. Using a digital pH meter, the carcass pH was determined in the *longissimus thoracis* (LT) between the 12th and 13th ribs at 24h postmortem. Meat colour parameters L* (lightness), a* (redness), and b* (yellowness) were assessed with a spectrocolorimeter (Konica Minolta CM508d, Minolta Co., Ltd, Osaka, Japan) under illuminant D65 as the light source. Following calibration with a standard white plate, the device was programmed to take three measurements and calculate their mean. Colour measurements were repeated three times for each *longissimus thoracis* et lumborum (LTL) sample after 24h of storage at 4°C, targeting the fat-free cut surface, and the mean of these readings was used as the final value. MLD Area, calculated by making a transverse cut between the 12th and 13th ribs, the surface at the level of the 12th rib was first drawn on acetate paper, and the area calculation was carried out using a planimeter. To determine cooking loss (CL), meat samples were initially weighed (initial sample weight), vacuum-packed, and cooked in a water bath at 75 °C for one h. After cooking, the samples were reweighed (cooked sample weight). Cooking loss (%) was calculated as the percentage weight reduction of the cooked sample relative to the initial sample weight, using the formula: (initial sample weight – cooked sample weight) / initial sample weight × 100 (37). The cooked samples were used to determine the shear force value after assessing the cooking loss. Six rectangular subsamples (3 cm in length and 1 × 1 cm in cross-sectional area), cut parallel to the muscle fibres, were obtained from the cooked samples and sheared across their widest dimension using a Universal Testing Machine (Zwick/Roell Z0.5, Ulm, Germany) equipped with a V-shaped Warner–Bratzler attachment (60º triangular aperture). The mean of the measurements from the six subsamples was recorded as the Warner–Bratzler shear force (WBSF) value for that sample (38). Water holding capacity (WHC) was determined using a modified Grau and Hamm method. Approximately 5 g of finely minced lean meat strips (cut parallel to muscle fibers) were placed between two pre-weighed filter papers, positioned between glass Petri dishes, and pressed with a 2.25 kg weight for 5 min. WHC (%) was calculated as the difference between the final and initial filter paper weights, divided by the sample weight, and multiplied by 100 (39).

Genetic Analysis

The gene polymorphisms analyzed in this study were g.1911A>G in intron 2 of the *MYF5* gene, A80V in exon 3 of the *LEP* gene, and C472T in the 5'UTR of the *IGF*1 gene. Blood samples (~4 mL) were collected from the *vena jugularis* of 103 Holstein-Friesian cattle into EDTA tubes. The Thermo Scientific GeneJET Genomic DNA Purification Kit was used according to the Mammalian Blood Genomic DNA Purification Protocol for DNA isolation. DNA concentration (ng/mL) and absorbance ratio at 260/280 were measured using a Nanodrop microvolume spectrophotometer (NanoDrop 2000c, Thermo Fisher Scientific, Wilmington, DE, USA).

The genotypes of the chosen loci were identified using the PCR-RFLP technique. Table 1 displays the primers, restriction enzymes and PCR conditions. The specificity of the primer sequences was verified using the NCBI GenBank database. The DNA amplification reactions were performed using a thermal cycler (Palm Cycler GC1-96, Corbett Research, Sydney, Australia). The PCR amplification was performed in a total volume of 50 µL containing

Table 1:The polymorphisms in the current study were genotyped using restriction enzymes, PCR conditions, and primer sequences (from 5´ to 3´).

Marker Name	Allele	Amplicon (bp)	Primers	PCR Conditions	Restriction Enzyme	Reference
LEP A80V	C/T	458	F: 5´GGGAAGGGCAGAAAGATAG3´ R: 5´CCAAGCTCTCCAAGCTCTC3´	94 °C 2' (94 °C 30 s, 57 °C 1' 72 °C 30 s) 35 cycles, 72 °C 15'	*Hph*I	(10)
IGF1 C472T	C/T	249	F: 5´ATTACAAAGCTGCCTGCCCC3´ R:5´ACCTTACCCGTATGAAAGGAATATACGT 3´	94 °C 5' (94 °C 1'64 °C 1', 72 °C 1') 31 cycles, 72 °C 5'	*Sna*BI	(29)
MYF5 g.1911A>G	A/G	445	F:5´ACAGCGTCTACTGTCCTGATG3´ R:5´CGTGGTATATACTAAGGACAC3´	94 °C 4' (94 °C 30 s, 58 °C 1', 72 °C 1') 38 cycles, 72 °C 4'	*Taq*I	(35)

36 µL of dH2O, 5 µL of 10X buffer, 3 µL of MgSO4, 1 µL of dNTPs (2.5 mM), 2.5U Taq DNA polymerase (Biomatik, A1003-500U, 5U/µL), 1 µL (0.025 µM) of each primer, and 2 µL of the DNA sample at a concentration of 100 ng/µL. After amplification, 15 µL of the PCR product with each SNP was digested in 15 units of the corresponding restriction enzyme. These reactions were incubated at 37°C for 16h. The digestion products were then electrophoresed in a 3% agarose gel (Sigma-Aldrich, Steinheim, Germany) at 85–90V for 1h. The gels were photographed using a gel-imaging system (DNR-Minilumi, DNR Bio-Imaging Systems, Israel).

Statistical Analysis

Genotypic and allelic frequencies were calculated according to Falconer and Mackay (40). The chi-square (v2) test established each SNP's Hardy-Weinberg equilibrium. The polymorphism information content (PIC), heterozygosity (He), number of effective alleles (Ne), and population genetics parameters were computed by Nei and Roychoudhury (41) and Botstein et al. (42). The following statistical models were used to examine the effects of genotypes on the variables under study using the least-squares methods as performed in a general linear model (GLM) process of Minitab (MINITAB®, USA, v17.1.0).

$$Y_{ijklmn} = \mu + S_i + W_j + AG_k + BG_l + CG_m + I_n + e_{ijklmn}$$

Where Y_{ijklmn} = phenotypic traits; µ= is the overall mean, Si= the fixed effect of season at the slaughter (i = winter and autumn), W_j = the fixed effect of age at slaughter (j = 14–17 months), AG_k = the fixed effect of the *MYF5* genotype for the g.1911A>G (k= AA, AG, GG), BG_l = the fixed effect of the *LEP* genotype for the A80V (l: CC, CT, TT), CG_m = the fixed effect of the *IGF*1 genotype for the C472T (m=CC, CT, TT), I_n = is the fixed effect of the genotypic interactions and eijklmn = random error.

Furthermore, to test statistical epistasis between the *LEP* and *MYF5* loci, each phenotype was analyzed with a two-way fixed-effects ordinary least-squares (OLS) model treating genotypes as categorical factors and including their interaction:

$$\gamma=\mu+C(LEP)+C(MYF5)+C(LEP)\times C(MYF5)+\varepsilon$$

Because frequencies were unbalanced across genotype combinations, Type-II sums of squares were used. The *LEP* × *MYF5* interaction term provided the test of epistasis; two-sided P-values are reported. As an effect size, we calculated partial eta-squared for the interaction,

$$\eta^2_{partial} = \frac{SS_{interaction}}{SS_{interaction} + SS_{residual}}$$

To control multiplicity across traits, P-values were adjusted using the Benjamini–Hochberg false discovery rate (FDR) at q=0.05. Because carcass traits (LW, HCW, CCW, DP) were specified a priori, FDR was also controlled within this subset and reported alongside the global FDR.

In the raw dataset, *MYF5* genotypes were encoded as AA, GA, and GG for analysis and visualization. Given sparse counts in the AA category, we performed sensitivity analyses under dominant genetic models: for *MYF5* (A-dominant: AA+GA vs GG; G-dominant: GG+GA vs AA) and for *LEP* (C-dominant: CC+CT vs TT; T-dominant: TT+CT vs CC), each evaluated with one-way OLS and reported with mean ± SE. Interaction plots display mean ± SE for each *LEP* × *MYF5* combination.

$$z_{i,t} = \frac{\bar{X}_{i,t}}{\sigma_t}$$

To jointly summarize carcass performance and meat-quality readouts across genotype combinations, we constructed composite scores on the (*LEP*, *MYF5*, *IGF*1) strata using a complete-case dataset (animals with non-missing values for LW, HCW, CCW, DP, WBSF, CL, WHC, pH, and L*). For each genotype combination, trait means were computed, and then standardized across combinations to z-scores: where $X_{i,t}$ is the mean of trait t in combination i, and µt, σt are the across-combination mean and SD, respectively. Concerning the carcass score, we summarized carcass performance as the unweighted average of z-scores for LW, HCW, CCW, and DP:

$$Carcass_i = \frac{1}{4}(z_{i,LW} + z_{i,HCW} + z_{i,CCW} + z_{i,DP})$$

For the quality score, directionality was aligned so that higher values indicate better quality. Specifically, lower is preferable for WBSF and CL, for which we used −z; higher is preferable for WHC, for which we used z. pH desirability was modeled as closeness to 5.6 using a quadratic penalty $d_{i,pH} = -(X_{i,pH} - 5.6)^2$, which was then standardized across combinations to z(dpH). L* was incorporated with a small weight to avoid over-emphasis; we formed a mild centrality score $d_{i,L} = -(X_{i,L} - \mu L)^2$ and standardized it to

z(dL).

The beef quality score was then:

$$Beef\ quality_i = ((-z_{i,WBSF}) + (-z_{i,CL}) + (z_{i,WHC}) + (z_{i,PH})) \times 0.9 + z(d_{i,L}) \times 0.1$$

Overall performance was summarized by linear combinations of the two sub-scores:

$$Combined_{50:50,i} = 0.50 \times Carcass_i + 0.50 \times Quality_i$$

$$Combined_{60:40,i} = 0.60 \times Carcass_i + 0.40 \times Quality_i$$

To characterize trade-offs between carcass and quality, we identified non-dominated combinations on the (Carcass, Quality) plane. Combination A was said to dominate B if CarcassA ≥ CarcassB and QualityA ≥ QualityB with at least one strict inequality. Combinations not dominated by any other were labeled Pareto_front = True (best attainable trade-offs).

Results

Genotypic variation and population genetics evaluation

Allele and genotype frequencies, as well as population genetic parameters of their selected polymorphisms, are shown in Table 2. Three potential genotypes were noted for each marker. While the highest allele frequency in the population was observed in the *MYF5*-G allele, the genotype with the highest number of individuals in the population was the *IGF*1-CT genotype. Among these three *Candida*te genes, only the genotypic distribution in the *MYF5* was consistent with the Hardy-Weinberg equilibrium. The chi-squared test showed that the SNPs of the *IGF*1 and *LEP* genes did not fit the HWE requirements. In evaluating population genetic parameters, only the *IGF*1 marker had a high Ne level among the selected markers (1.93). The population is moderately informative for all three selected markers. (0.25<PIC<0.50).

Table 2: A Holstein population's gene frequencies, population genetic indices, and HWE test of polymorphisms in the *IGF1*, *LEP*, and *MYF5* genes.

	LEP A80V			*IGF1* C472T			*MYF5* g.1911A>G		
Genotypes	CC	CT	TT	CC	CT	TT	AA	AG	GG
N	16	30	57	11	62	30	4	49	50
Genotype frequency (%)	15.5	29.1	55.4	10.7	60.2	29.1	3.9	47.6	48.5
EGF (%)	9.3	43.3	50.3	17.1	49.7	36.1	7.9	41.2	53.9
Alleles	C	T		C	T		A	G	
Allele frequency	0.30	0.70		0.41	0.59		0.28	0.72	
He	0.42			0.48			0.40		
Ne	1.73			1.93			1.67		
PIC	0.332			0.366			0.320		
X^2(HWE)	9.76			6.25			3.66		
P	0.002**			0.012**			0.056*		

EGF: expected genotype distribution according to HWE; He: heterozygosity; Ne: effective allele number; PIC: polymorphism information content; x^2(HWE): Hardy–Weinberg equilibrium x^2 value. *P > 0.05: consistent with HWE.**P<0.05: not consistent with HWE.

Effects of the markers on traits

The relationships identified in this investigation regarding the individual effects of the selected genetic markers on trait means are summarized in Table 3. In this context, no significant relationship was observed between *LEP*, *IGF*1, and *MYF5* markers and carcass quality traits (P>0.05). Tables 4 and 5 present the levels of least-squares means and standard errors for the impacts of *MYF5* g.1911A>G, *IGF*1 C472T, and *LEP* A80V on carcass traits and meat quality. The study identified significant interactions within the carcass quality using genotypic interaction analysis. *LEP* × *MYF5* gene interaction was significantly associated with live weight, hot carcass weight, and chilled carcass weight traits (P<0.05). The CCAA genotypes for live weight, hot carcass weight, and CCAG genotypes for chilled carcass weight seem favorable in the *LEP* × *MYF5* interactions.

Table 3: An overview of the associations observed.

Phenotypic Traits	Genotype			
	LEP	*MYF5*	*IGF1*	*LEP* × *MYF5*
Live weight, kg	0.557	0.179	0.926	**0.002**
Hot carcass weight, kg	0.460	0.154	0.931	**0.008**
Dressing percentage, %	0.146	0.783	0.413	0.844
Chilled carcass weight, kg	0.468	0.166	0.918	**0.009**
pH	0.943	0.353	0.614	0.292
*L** (lightness)	0.632	0.771	0.637	0.774
*a** (redness)	0.767	0.307	0.155	0.795
*b** (yellowness)	0.869	0.384	0.871	0.612
Carcass lenght, cm	0.150	0.593	0.527	0.897
Chest width, cm	0.491	0.923	0.567	0.734
MLD area	0.936	0.543	0.561	0.865
WBSF, kgf/cm^2	0.179	0.683	0.571	0.894
Cooking loss, %	0.861	0.647	0.777	0.789
Water holding capacity	0.259	0.704	0.943	0.674
Chilling loss, %	0.596	0.379	0.493	0.262

Table 4: The impact of *IGF1*, *LEP*, and *MYF5* on carcass traits and meat quality in a Holstein population was measured using least-squares means and standard errors.

Marker	Genotype	Traits									
		LW	HCW	CCW	DP	pH	MLD	WBSF Slop	CL	CHL	WHC
LEP	CC	473.1 ± 25.4	255.8 ± 15.5	251.8 ± 15.3	53.97 ± 1.19	5.55 ± 0.13	94.04 ± 8.42	3.50 ± 0.85	26.09 ± 2.55	1.58 ± 0.18	11.88 ± 1.30
	CT	442.2 ± 17.9	232.0 ± 10.9	228.7 ± 10.8	52.29 ± 0.84	5.57 ± 0.10	94.2 ± 5.93	5.53 ± 0.59	26.38 ± 1.81	1.41 ± 0.13	13.50 ± 0.68
	TT	465.9 ± 22.4	236.2 ± 13.6	232.4 ± 13.5	50.96 ± 1.05	5.52 ± 0.12	97.13 ± 7.41	4.95 ± 0.77	24.91 ± 2.25	1.59 ± 0.16	11.92 ± 0.91
IGF1	CC	456.5 ± 22.6	238.9 ± 13.8	235.0 ± 13.6	52.19 ± 1.06	5.56 ± 0.12	90.68 ± 7.49	4.43 ± 0.76	26.48 ± 2.26	1.58 ± 0.16	12.46 ± 1.54
	CT	464.2 ±13.5	241.1 ± 8.20	237.3 ± 8.09	51.98 ± 0.63	5.58 ± 0.07	98.24 ± 4.46	5.04 ± 0.47	25.97 ± 1.37	1.57 ± 0.10	12.27 ± 0.67
	TT	460.4 ± 16.7	244.1 ± 10.1	240.6 ±10.0	53.06 ± 0.78	5.49 ± 0.09	96.45 ± 5.51	4.51 ± 0.59	24.92 ± 1.69	1.43 ± 0.12	12.56 ± 0.78
MYF5	AA	447.0 ± 33.2	229.8 ± 20.2	226.6 ± 19.9	51.68 ± 1.56	5.50 ± 0.18	90.4 ± 11.0	4.10 ± 1.10	27.46 ± 3.32	1.37 ± 0.23	12.82 ± 1.57
	AG	482.5 ± 11.5	256.5 ± 7.03	252.2 ± 6.9	52.81 ± 0.54	5.63 ± 0.06	100.1 ± 3.82	5.08 ± 0.39	25.44 ± 1.17	1.66 ± 0.08	11.96 ± 0.65
	GG	451.6 ± 13.8	237.7 ± 8.39	234.1 ± 8.3	52.73 ± 0.65	5.50 ± 0.07	94.86 ± 4.56	4.80 ± 0.49	24.48 ±1.38	1.54 ± 0.10	12.51 ± 0.65
LEP × *MYF5*	CCAA	480.5 ± 67.5^{abc}	260.0 ± 41.1^{ab}	257.0 ± 40.5^{ab}	54.62 ± 3.16	5.53 ± 0.36	89.1 ± 22.3	1.99 ± 2.24	27.91 ± 6.75	1.16 ± 0.47	12.80 ± 2.55
	CCAG	516.0 ± 23.3^{a}	279.9 ± 14.2^{a}	274.6 ± 14.0^{a}	53.76 ± 1.09	5.74 ± 0.13	100.9 ± 7.71	4.57 ± 0.78	27.27 ± 2.38	1.91 ± 0.16	12.98 ± 0.88
	CCGG	422.8 ± 28.6^{d}	227.5 ± 17.4^{c}	223.8 ± 17.2^{c}	53.53 ± 1.34	5.39 ± 0.16	92.13 ± 9.45	3.93 ± 0.95	23.10 ± 2.85	1.65 ± 0.20	12.27 ± 1.08
	CTAA	372.1 ± 42.8^{e}	188.2 ± 26.0^{d}	185.9 ± 25.7^{d}	50.76 ± 2.01	5.59 ± 0.24	83.8 ± 14.1	5.12 ± 1.43	27.49 ± 4.28	1.18 ± 0.30	14.59 ± 1.61
	CTAG	494.9 ± 20.4^{ab}	264.7 ± 12.4^{ab}	260.5 ± 12.3^{ab}	53.36 ± 0.96	5.62 ± 0.11	102.4 ± 6.8	5.67 ± 0.68	25.32 ± 2.06	1.61 ± 0.14	13.66 ± 0.77
	CTGG	459.5 ± 16.5^{bc}	243.1 ± 10.0^{b}	239.6 ± 9.9^{b}	52.75 ± 0.77	5.48 ± 0.09	96.39 ± 5.45	5.80 ± 0.57	26.32 ± 1.70	1.44 ± 0.12	12.66 ± 0.62
	TTAA	488.4 ± 54.2^{ab}	241.3 ± 33.0^{bc}	236.9 ± 32.6^{bc}	49.66 ± 2.54	5.38 ± 0.29	98.3 ± 17.9	5.19 ± 1.83	26.97 ± 5.43	1.77 ± 0.38	12.44 ± 2.05
	TTAG	436.7 ± 15.9^{cd}	224.8 ± 9.7^{c}	221.5 ± 9.6^{c}	51.30 ± 0.75	5.54 ± 0.09	97.02 ± 5.26	4.99 ± 0.57	23.73 ± 1.64	1.47 ± 0.11	11.84 ± 0.60
	TTGG	472.5 ± 17.6^{b}	242.6 ± 10.7^{b}	238.9 ± 10.6^{b}	51.92 ± 0.83	5.63 ± 0.09	96.07 ± 5.84	4.67 ± 0.68	24.02 ± 1.80	1.53 ± 0.12	11.78 ± 0.67

LW: Live weight; HCW: Hot carcass weight; CCW: Chilled carcass weight; DP: Dressing percentage; MLD: Musculus Longissimus dorsi. WBSF Slop: Warner Bratzler shear force; CL: Cooking loss; CHL: Chilling loss; WHC: Water holding capacity.

^{a,b,c,d} Means with different superscripts differ significantly.

Table 5: The impact of *IGF1*, *LEP*, and *MYF5* on carcass measurement and meat colour in a Holstein population was measured using least-squares means and standard errors.

Marker	Genotype	Traits				
		CLE	CW	*L**	*a**	*b**
LEP	CC	140.35 ± 1.28	78.59 ± 2.03	34.75 ± 2.48	10.18 ± 1.22	9.47 ± 2.00
	CT	138.59 ± 0.93	79.72 ± 1.43	36.06 ± 1.74	11.19 ± 0.88	9.19 ± 1.45
	TT	141.35 ± 1.13	81.53 ± 1.78	33.48 ± 2.18	10.42 ± 1.08	10.32 ± 1.76
IGF1	CC	139.30 ± 1.14	78.83 ± 1.80	36.05 ± 2.20	9.28 ± 1.09	10.21 ± 1.79
	CT	140.39 ± 0.68	80.13 ± 1.07	34.29 ± 1.31	11.18 ± 0.65	9.55 ± 1.06
	TT	140.6 ± 0.84	80.87 ± 1.33	33.94 ± 1.62	11.33 ± 0.80	9.22 ± 1.32
MYF5	AA	141.06 ± 1.68	79.24 ± 2.64	33.28 ± 3.23	11.73 ± 1.60	11.88 ± 2.63
	AG	139.35 ± 0.59	80.31 ± 0.92	35.70 ± 1.12	9.53 ± 0.56	8.94 ± 0.92
	GG	139.89 ± 0.71	80.29 ± 1.10	35.30 ± 1.34	10.53 ± 0.67	8.16 ± 1.10
LEP × *MYF5*	CCAA	141.27 ± 3.40	74.89 ± 5.37	30.72 ± 6.56	12.04 ± 3.24	12.75 ± 5.31
	CCAG	139.52 ± 1.23	80.34 ± 1.85	37.66 ± 2.27	8.31 ± 1.17	9.15 ± 1.92
	CCGG	140.27 ± 1.48	80.55 ± 2.27	35.85 ± 2.78	10.19 ± 1.41	6.51 ± 2.31
	CTAA	138.6 ± 2.27	79.38 ± 3.40	36.27 ± 4.16	13.05 ± 2.17	8.57 ± 3.56
	CTAG	138.22 ± 1.05	80.33 ± 1.63	36.33 ± 1.99	10.01 ± 0.1	9.64 ± 1.64
	CTGG	138.96 ± 0.83	79.43 ± 1.31	35.57 ± 1.60	10.52 ± 0.79	9.36 ± 1.30
	TTAA	143.31 ± 2.73	83.44 ± 4.32	32.84 ± 5.28	10.11 ± 2.61	14.31 ± 4.28
	TTAG	140.30 ± 0.84	80.25 ± 1.27	33.12 ± 1.55	10.26 ± 0.80	8.03 ± 1.31
	TTGG	140.44 ± 0.89	80.89 ± 1.40	34.48 ± 1.72	10.89 ± 0.85	8.61 ± 1.39

CLE: Carcas length; CW: Chest width; *L* (lightness). a* (redness). b* (yellowness)*

Discussion

Genetic improvement studies have shown that certain desirable traits can be enhanced through selective breeding. This has also raised concerns about decreased genetic diversity and increased risk of inbreeding, demonstrating the need for a balanced approach in breeding programs (43). Furthermore, the economic impact of these genetic improvements is profound, with the potential to significantly increase the market value of beef products and improve sustainability in livestock production. The integration of advanced genomic techniques into breeding programs, along with ongoing research into the genetic mechanisms underlying these relationships, offers opportunities to optimize carcass quality and meet the evolving demands of the beef industry. Understanding these genetic influences is crucial for developing robust breeding strategies that improve meat quality while considering economic viability and ecological sustainability in cattle production. *LEP*, *IGF*1 and *MYF5* genes are involved in different but interrelated metabolic pathways that affect carcass composition and meat quality in cattle. While the *LEP* gene affects energy balance, appetite control and fat metabolism through the *LEP*tin hormone released from adipocytes, *IGF*1 plays an active role in musculoskeletal development, cell proliferation, and protein synthesis processes through growth hormone (44, 45). The interaction of these two genes is crucial in coordinating energy use and fat storage, particularly in muscle tissue. Studies examining these relationships in livestock animals have been conducted (46-49). On the other hand, *MYF5* is the first myogenic regulatory factor that initiates the differentiation of muscle progenitor cells in the embryonic period and determines the number

and structure of muscle fibers. While *IGF*1 promotes muscle cell growth by influencing the expression of the *MYF5* gene, *LEP*tin hormone levels indirectly modulate these processes according to energy availability (50). Therefore, these three genes form a functional network in muscle mass formation (myogenesis), fat distribution and metabolic energy management. These relationships suggest that multi-gene interactions should be considered in genetic selection programs, particularly for carcass quality and meat yield. In this context, this study evaluated the effects of several pleiotropic genes, which play important roles in influencing various phenotypic traits, on carcass and meat quality traits in the Holstein–Friesian cattle breed.

To evaluate genotype-phenotype interactions in the population, genotype diversity and distribution must first be checked (51). If the minor allele frequency of a genetic marker is below 5%, it should be ignored because it is thought that the relevant marker will not provide reliable results in the population (52). In this study, the minor allele frequency was calculated to be between 0.28 and 0.41. The results are sufficient for subsequent association analyses. Population genetic parameters (He, Ne, PIC) help evaluate population structure based on the genetic variation of the genes involved. Low heterozygote values provide important data for pedigree evaluation, such as inbreeding, by interpreting the prevalence of genotypes within the population. Ne is used to express the effect of locus alleles in populations. In this study, the He values ranged from 0.40 to 0.48, while the Ne values ranged from 1.67 to 1.93. In addition to reflecting the quantity of detectable alleles and their distribution throughout a population, PIC values offer a quantitative assessment of a marker's informativeness. A marker with a higher PIC value is more useful for genetic research, as it exhibits greater allelic diversity and a more balanced allele frequency distribution. According to Botstein et al., polymorphisms have three main informativeness levels: low-informative polymorphism for PIC < 0.25, moderate-informative polymorphism for 0.25 < PIC < 0.50, and high-informative polymorphism for PIC > 0.50. It can be interpreted that all markers have a moderate level of informativeness.

ANOVA results show no significant influences of *LEP*, *IGF*1 and *MYF5* on carcass traits and meat quality. Studies in the literature examined the relationship between different *LEP* gene polymorphisms and cattle carcass quality (53, 55). The A80V polymorphism was detected as monomorphic in the Japanese Brown Cattle breed, and the C allele was reported to be dominant in the population (56). The results of this study indicate that the Holstein-Friesian cattle population is polymorphic, with the T allele being more prevalent in the population. In the study examining the carcass quality and lipid profile of the *LEP* gene in Nellore breed cattle, the relationship between the cold carcass weight, L*, a*, b*, CL, and WBSF (kgf), which are the targeted traits in this study, was examined. However, no statistically significant result was obtained (P>0.05) (57). In a study conducted on Holstein cattle, the relationship between the *LEP* gene variations and hot carcass weight, dressing percentage, chilled carcass weight, carcass length, chest width, and meat color parameters was examined (23). However, no significant relationship was found (P>0.05). In Simmental cattle, a significant relationship was found between hot and chilled carcass weight and dressing percentage (P<0.05) (4). In this study conducted on Holstein-Friesian cattle, no significant association was found between the *LEP* gene and carcass and meat quality traits. The results further demonstrate that these association analyses were conducted on a population-specific basis. The *LEP* A80V polymorphism is a widely studied marker for both milk yield and fattening performance traits. As a crucial marker for meat quality in cattle, studying *LEP* A80V in different breeds and repeating these studies in various populations will contribute to effective selection programs.

The *IGF*1 and *MYF5* genes are located in BTA5, which contains quantitative trait loci (QTL) affecting important traits such as milk yield, growth and development in the cattle genome. For the *IGF*1 gene, the literature has observed an increase in studies related to growth traits (58, 59), meat quality (60), and carcass traits (61) in small ruminants. Studies have been conducted on different breeds to improve carcass quality in cattle (62, 63). The relationship between *IGF*1 C472T polymorphism and meat color, tenderness and cooking loss traits was investigated in Holstein-Friesian cattle. It was found to be associated with a* color parameter value 24 hours after death (P < 0.05) (30). This study examined the relationship with meat color parameters, but no significant results were obtained. In the study conducted on Turkish Grey Steppe, Anatolian Black, and East Anatolian Red cattle breeds, it was reported that the T allele was dominant in all three breeds for the *IGF*1 C472T polymorphism, and the number of individuals with the TT genotype was high (64). In this study, the T allele is relatively dominant compared to the C allele, and the number of individuals with the heterozygous genotype is the highest in the population. The *IGF*1 C472T polymorphism has been investigated for growth traits in Kazakh white-headed cattle (65) and milk production traits in Holstein–Friesian cattle (66). This suggests that the *IGF*1 C472T polymorphism is a significant marker for develop-

ing economic traits in cattle, although no significant results were obtained in this study. The role of the *MYF5* gene in myogenesis places it in an important position for traits that are primary targets in breeding, such as meat and carcass quality, and fattening performance. There are current relationship analysis studies in small ruminants (67), buffalo (68) and even camels (69). Classification and regression tree (CART) analysis reported that the *MYF5* gene was associated with fattening performance in Brown Swiss and Holstein cattle breeds (70). A study conducted on the Turkish Grey Steppe, Anatolian Black, and East Anatolian Red cattle breeds reported that only the East Anatolian Red cattle breed exhibited the A allele as the dominant allele in the population. In contrast, the G allele was dominant for the other two breeds, and even the A allele was not found in the Anatolian Black breed (64). In the study in which a total of 108 animals, including Angus, Angus×Hereford×Nellore, Brahman, Hereford, Limousine, and Charolais breeds, were genotyped, the A allele for the *MYF5* g.1911A>G polymorphism was found only in Angus×Hereford×Nellore crossbreds and Hereford breeds. However, the number of individuals with the AA genotype was not more than five (71). The results of this study examining the Holstein-Friesian population are supportive, and the G allele is quite dominant in the population. The relationship between *MYF5* g.1911A>G polymorphism and meat color, tenderness and cooking loss traits was investigated in Holstein-Friesian cattle. It was found to be associated with a* color parameter (P<0,05) (30). Although the present study evaluated associations with meat color parameters, no statistically significant relationships were observed.

Our data indicate that the *LEP* × *MYF5* interaction is associated with variation in carcass performance (most notably LW, HCW, and CCW), whereas no corresponding interaction was detected for meat-quality endpoints. Effect sizes for the interaction were modest but non-trivial (partial $\eta^2 \approx 0.10–0.13$), consistent with a non-additive interplay between endocrine regulation of energy balance (*LEP*tin signaling) and myogenic programming (*MYF5*) that primarily influences growth and carcass composition rather than post-mortem quality attributes. The absence of interaction effects on pH, color, WHC, CL, or WBSF suggests that downstream biochemical processes governing tenderness and moisture retention are less sensitive to this particular gene–gene combination, at least within the production context studied.

A complementary multi-criteria framework that jointly summarized carcass yield and meat quality supported these conclusions (Figure 1). When carcass traits were prioritized, the *LEP*-CC × *MYF5*-GA combination ranked highest, with *LEP*-TT × *MYF5*-GG emerging as a robust, well-represented alternative. In contrast, for meat quality, *IGF*1-CT tended to show a favorable profile (lower Warner–Bratzler shear force and cooking loss with comparable water-holding capacity). Taken together, these results suggest that genotype-aware decision-making should balance yield and quality (e.g., incorporating combination effects, rather than relying on single-locus markers alone) in selection indices or mating plans. Sensitivity analyses under dominant genetic models yielded consistent patterns, further supporting the stability of the main inferences.

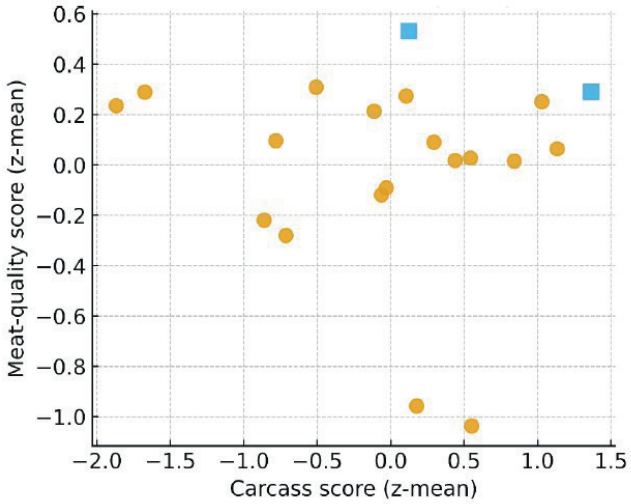


Figure 1. Trade-off between carcass and meat quality (Pareto highlighted). Each point represents a (*LEP*. *MYF5*. *IGF1*) genotype combination. X-axis: Carcass score (equal-weighted mean of across-combination z-scores for LW, HCW, CCW, and DP). Y-axis: Quality score (z with directionality: lower-is-better for WBSF and CL [sign-reversed], higher-is-better for WHC [z]. pH desirability modeled as closeness to 5.6 and standardized; L* included with a small weight). Higher values on both axes indicate better performance. Squares denote Pareto-optimal (non-dominated) combinations; circles denote dominated solutions. For each combination. we report the minimum contributing sample size across the included traits (min_n); combinations with min_n < 5 were flagged (low_n_flag) for cautious interpretation.

LW: live weight; HCW: hot carcass weight; CCW: chilled carcass weight; DP: dressing percentage; WBSF: Warner–Bratzler shear force; CL: cooking loss; WHC: water-holding capacity.

This study has limitations due to unbalanced genotype frequencies and the rarity of the *MYF5*-AA genotype, which increases uncertainty for cells involving AA and warrants cautious interpretation. The cohort comprised Holstein–Friesian bulls from a single production system; generalization to other sexes, management conditions, or breeds requires replication. Although we controlled error rates across multiple traits, residual confounding (e.g., subtle

management or slaughter-age variation) cannot be entirely excluded, and causal variants or regulatory haplotypes underlying the observed statistical epistasis were not mapped. Future work should validate these signals in independent populations, test gene-by-environment interactions, and integrate fine-mapping or functional assays to resolve the biological mechanisms. In short, our findings highlight an epistatic architecture in which *LEP* and *MYF5* jointly modulate carcass yield, with minimal impact on standard meat-quality endpoints, and point toward practical opportunities to exploit genotype combinations (alongside *IGF*1) in breeding programs that explicitly trade off carcass performance against quality.

Although the Holstein-Friesian breed is widely grown worldwide due to its high milk yield potential, the use of male calves as fattening material and the improvement of the meat quality of dairy cows after slaughter have made carcass traits necessary. LW, HCW, and CCW in cattle are the most fundamental yield parameters that determine the economic value of meat production. LW reflects the efficiency of the fattening process and growth rate, while hot and chilled carcass weights directly indicate the amount of marketable meat and carcass yield (72). Furthermore, achieving optimal quality will entirely depend not only on the quality of the pre- and post-slaughter process but also on the molecular mechanisms (73-76). In this study, when the individual effects of *LEP* and *MYF5* genes were examined, no significant relationship was found with the carcass traits in question. However, the *LEP* × *MYF5* gene interaction was found to be statistically significant for live weight, hot carcass weight, and chilled carcass weight. This suggests that gene-gene interactions (epistatic effects) may be important in phenotypes where the effects of individual genes are weak or unobservable. The interaction of these two genes can potentially affect carcass weight by jointly regulating muscle mass and fat distribution. This finding suggests that genetic studies to improve carcass yield in cattle should focus not only on individual gene effects but also on gene–gene interactions, thus providing a new contribution to the literature on the role of epistatic effects on economically important carcass traits.

Although *IGF*1 was included in the present study as a biologically plausible *Candida*te gene, the lack of significant associations with carcass or meat-quality traits may be explained by several factors. First, previous work in Turkish cattle populations has shown that the C472T polymorphism of *IGF*1 often displays low minor-allele frequencies and limited population variability, which reduces statistical power for detecting phenotypic effects (e.g., near fixation of the major allele in Holstein crosses) (77). Second, the C472T variant is located in the 5'-non-coding regulatory region rather than a coding exon, suggesting that its functional impact may be subtle, context-dependent, or detectable only under specific physiological states such as early growth or metabolic stress. Third, breed-specific transcriptional regulation of *IGF*1 has been documented across dairy and beef breeds, where differential expression rather than sequence variation drives phenotypic divergence, as emphasized in studies of milk-production pathways involving growth-factor regulation (78). Finally, gene–environment interactions (including nutritional regime, metabolic load, and management conditions) may modulate *IGF*1 signaling, thereby obscuring direct genotype–phenotype correlations. Collectively, these considerations suggest that while *IGF*1 remains mechanistically relevant to muscle accretion, its 5'UTR polymorphism alone may be insufficient to explain phenotypic variation in Holstein-Friesian bulls, especially in populations with restricted allelic diversity.

From an applied breeding perspective, the demonstrated *LEP* × *MYF5* interaction underscores the importance of incorporating epistatic marker combinations, rather than single-locus effects, into selection strategies targeting carcass performance. Similar to recent genomic-selection studies in dairy breeds, where composite genomic indices, haplotype interactions, and pathway-level markers have improved selection accuracy for milk yield, milk composition, and metabolic traits (78-81), the present findings suggest that multi-gene models may also enhance the prediction of carcass traits in dairy-type beef production systems. In production environments such as Türkiye, where male Holstein calves constitute a substantial share of the beef supply, identifying advantageous genotype combinations (e.g., *LEP* × *MYF5*) could support more efficient marker-assisted selection or genomic-index weighting to improve carcass weight and yield without compromising meat quality endpoints. Ultimately, integrating epistatic information into breeding programs may contribute to more precise genetic improvement, better resource use, and higher economic returns in dairy-origin beef production systems.

Conclusion

Live weight, hot carcass weight, and chilled carcass weight are the primary performance criteria that directly impact both production economy and carcass yield. Although *LEP* and *MYF5* genes did not show significant associations with the traits in question in individual analyses, the *LEP* × *MYF5* gene interaction was found to be statistically significant in these carcass parameters. The interaction of these

two genes may optimize carcass weight by co-regulating muscle mass and fat distribution in the Holstein-Friesian breed. This suggests that epistatic effects in the Holstein-Friesian breed may reveal genetic mechanisms that cannot be detected at the single-gene level but contribute significantly to the phenotype when considered together. In this context, the findings reveal that epistatic marker combinations should be considered not only for the Holstein-Friesian breed but also in breeding strategies to improve meat yield traits of dairy breeds.

Acknowledgements

This study did not involve any invasive procedures in animals by the authors. Therefore, no specific ethics statement was required, as the blood samples were collected from the natural flow of exsanguination during routine slaughterhouse procedures. All procedures were conducted in full compliance with the Regulation on the Working Procedures and Principles of Animal Experiments Ethics Committees, published in the Official Gazette of the Republic of Türkiye (No. 28914, 15 February 2014). The authors gratefully acknowledge the support of Tabiat Agriculture Farm/ Özdilek (Bursa). Moreover, an oral presentation of some of the research's findings was presented at the 8th International Eurasian Conference on Biological and Chemical Sciences (EurasianBioChem) in 2025.

References

1. Utrera ÁR, Van Vleck LD. Heritability estimates for carcass traits of cattle: a review. Genetics and Molecular Research, 2004; 3(3), 380-394.
2. Casas E, White SN, Riley DG, et al. Assessment of single nucleotide polymorphisms in genes residing on bovine chromosome 5: associations with beef carcass traits. Journal of Animal Science, 2000; 78(5), 1224-1230.
3. Schenkel FS, Miller SP, Jiang Z, et al. Association of a single nucleotide polymorphism in the calpastatin gene with carcass and meat quality traits of beef cattle. Journal of Animal Science, 2006; 84(2), 291-299.
4. Ardicli S, Dincel D, Samli H, et al. Effects of polymorphisms at LEP, CAST, CAPN1, GHR, FABP4 and DGAT1 genes on fattening performance and carcass traits in Simmental bulls. Archives Animal Breeding, 2017a; 60(2), 61-70.
5. Liefers SC, Te Pas MFW, Veerkamp RF, et al. Associations between leptin gene polymorphisms and production, live weight, energy balance, feed intake, and fertility in Holstein heifers. Journal of Dairy Science, 2002; 85(6), 1633-1638.
6. Liefers SC, Veerkamp RF, Te Pas MFW, et al. Leptin promoter mutations affect leptin levels and performance traits in dairy cows 1. Animal Genetics, 2005; 36(2), 111-118.
7. Kumar S, Dahiya S, Magotra A, et al. Study of Polymorphism in exon 3 region of leptin Gene in Harnali sheep. The Pharma Innovation Journal, 2020; 9(2), 360-363.
8. Salgado Pardo JI, Delgado Bermejo JV, González Ariza A, et al. Candidate genes and their expressions involved in the regulation of milk and meat production and quality in goats (Capra hircus). Animals, 2022; 12(8), 988.
9. Banos G, Woolliams JA, Woodward BW, et al. Impact of single nucleotide polymorphisms in leptin, leptin receptor, growth hormone receptor, and diacylglycerol acyltransferase (DGAT1) gene loci on milk production, feed, and body energy traits of UK dairy cows. Journal of Dairy Science, 2008; 91(8), 3190-3200.
10. Oztabak K, Toker NY, Un C, et al. Leptin gene polymorphisms in native Turkish cattle breeds. Kafkas Üniversitesi Veteriner Fakültesi Dergisi, 2010; 16(6).
11. De Matteis G, Scatà MC, Grandoni F, et al. Association analyses of single-nucleotide polymorphisms in the leptin and leptin receptor genes on milk and morphological traits in Holstein cows. Open Journal of Animal Sciences, 2012; 2(3), 174-182.
12. Kawaguchi F, Okura K, Oyama K, et al. Identification of leptin gene polymorphisms associated with carcass traits and fatty acid composition in Japanese Black cattle. Animal Science Journal, 2017; 88(3), 433-438.
13. Ardicli S, Samli H, Vatansever B, et al. Comprehensive assessment of candidate genes associated with fattening performance in Holstein–Friesian bulls. Archives Animal Breeding, 2019a; 62(1), 9-32.
14. Prihandini PW, Hariyono DNH, Sari APZNL, et al. Association between GH, PRL, LEP, and PIT-1 gene polymorphisms and growth traits in Indonesian Rambon indigenous cattle. Tropical Animal Health and Production, 2025; 57(2), 56.
15. Singh SK, Rout PK, Agarwal R, et al. Characterization of exon 2 and intron 2 of leptin gene in Indian goats. Animal Biotechnology, 2009; 20(2), 80-85.
16. Korkmaz-Agaoglu O, Elmaz O, Akyuz B, et al. Identifying polymorphism in some genes and their effects on growth performance in Honamli and hair goat breeds. Genetika, 2019; 51(3), 995-1008.
17. Senturk N, Selvi TN, Demir M, et al. The impact of LEP gene polymorphisms located at exon 2 (LEP-HinfI) and intron 2 (LEP-Sau3AI) on growth and reproductive traits in Saanen goats. Archives Animal Breeding, 2024; 67(4), 523-531.

18. Stone RT, Kappes SM, Beattie CW. The bovine homolog of the obese gene maps to chromosome 4. Mammalian Genome, 1996; 7,399–400.
19. Nkrumah JD, Keisler DH, Crews Jr DH, et al. Genetic and phenotypic relationships of serum leptin concentration with performance, efficiency of gain, and carcass merit of feedlot cattle. Journal of Animal Science, 2007; 85(9), 2147-2155.
20. Giblin L, Butler ST, Kearney BM, et al. Association of bovine leptin polymorphisms with energy output and energy storage traits in progeny tested Holstein-Friesian dairy cattle sires. BMC Genetics, 2010; 11(1), 73.
21. Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. Nature, 1998; 395(6704), 763-770.
22. Yazdani H, Rahmani HR, Edris MA, et al. Association between A59V polymorphism in exon 3 of leptin gene and reproduction traits in cows of Iranian Holstein. African Journal of Biotechnology, 2010; 9(36).
23. Ardicli S, Samli H, Dincel D, et al. Individual and combined effects of CAPN1, CAST, LEP and GHR gene polymorphisms on carcass characteristics and meat quality in Holstein bulls. Archives Animal Breeding, 2017b; 60(3), 303-313.
24. Ge W, Davis ME, Hines HC, et al. Association of a genetic marker with blood serum insulin-like growth factor-I concentration and growth traits in Angus cattle. Journal of Animal Science, 2001; 79(7), 1757-1762.
25. Bishop MD., Tavakkol A, Threadgill DW, et al. Somatic cell mapping and restriction fragment length polymorphism analysis of bovine insulin-like growth factor I. Journal of Animal Science, 1991; 69(11), 4306-4311.
26. Miller JR, Thomsen PD, Dixon SC, et al. Synteny mapping of the bovine IGHG2, CRC and IGF1 genes. Animal Genetics,1992; 23, 51-58.
27. Li C, Basarab J, Snelling WM, et al. Assessment of positional candidate genes myf 5 and igf 1 for growth on bovine chromosome 5 in commercial lines of Bos taurus. Journal of Animal Science, 2004; 82(1), 1-7.
28. Curi RA, De Oliveira HN, Silveira AC, et al. Effects of polymorphic microsatellites in the regulatory region of IGF1 and GHR on growth and carcass traits in beef cattle. Animal Genetics, 2005; 36(1), 58-62.
29. Siadkowska E, Zwierzchowski L, Oprzadek J, et al. Effect of polymorphism in IGF-1 gene on production traits in Polish Holstein-Friesian cattle. Animal Science Papers and Reports, 2006; 24(3), 225-37.
30. Ardicli S, Samli H, Dincel D, et al. Relationship of the bovine IGF1, TG, DGAT1 and MYF5 genes to meat colour, tenderness and cooking loss. Journal of the Hellenic Veterinary Medical Society, 2018; 69(3), 1077-1087.
31. Weintraub H, Davis R, Tapscott S, et al. The myoD gene family: nodal point during specification of the muscle cell lineage. Science, 1991;251(4995):761–766.
32. Ujan J, Zan L, Ujan S, et al. Back fat thickness and meat tenderness are associated with a 526 T/A mutation in the exon 1 promoter region of the MyF-5 gene in Chinese Bos taurus. Genetics and Molecular Research, 2011;10(4):3070–3079.
33. Zhao C, Raza SHA, Khan R, et al. Genetic variants in MYF5 affected growth traits and beef quality traits in Chinese Qinchuan cattle. Genomics, 2020;112(4): 2804–2812.
34. Bhuiyan MSA, Kim NK, Cho YM, et al. Identification of SNPs in MYOD gene family and their associations with carcass traits in cattle. Livestock Science, 2009; 126(1-3), 292-297.
35. Kisacova J, Kubek A, Melus V, et al. Genetic polymorphism of Myf-5 and myostatin in Charolais breed. Journal of Agrobiology, 2009; 26: 7-11.
36. Sagsoz Y, Coban O., Lacin E, et al. Esmer ve Şarole x Esmer danaların besi performansı ve karkas özellikleri. Atatürk Üniversitesi Ziraat Fakültesi Dergisi, 2005; 36(2), 163-169.
37. Ekiz B, Yilmaz A, Ozcan M, et al. Carcass measurements and meat quality of Turkish Merino, Ramlic, Kivircik, Chios and Imroz lambs raised under an intensive production system. Meat Science, 2009; 82(1), 64-70.
38. Ekiz B, Ekiz EE, Kocak O, et al. Effect of pre-slaughter management regarding transportation and time in lairage on certain stress parameters, carcass and meat quality characteristics in Kivircik lambs. Meat Science, 2012; 90(4), 967-976.
39. Beriain MJ, Horcada A, Purroy A, et al. Characteristics of Lacha and Rasa Aragonesa lambs slaughtered at three live weights. Journal of Animal Science, 2000; 78(12), 3070-3077.
40. Falconer DS, Mackay TFC. Introduction to Quantitative Genetics, Harlow, Pearson Education Ltd., ISBN 0582243025,1996.
41. Nei M, Roychoudhury, A. Sampling variances of heterozygosity and genetic distance, Genetics, 1974; 76, 379–390.
42. Botstein D, White RL, Skolnick M, et al. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. American Journal of Human Genetics, 1980; 32(3), 314.
43. Lacorte GA, Machado MA, Martinez ML, et al. DGAT1 K232A polymorphism in Brazilian cattle breeds. Genetics and Molecular Research, 2006; 5(3), 475-482.

44. Houseknecht K, Baile CA, Matteri RL, et al. The biology of leptin: a review. Journal of Animal Science, 1998; 76(5), 1405-1420.
45. Florini JR, Ewton DZ, Coolican SA. Growth hormone and the insulin-like growth factor system in myogenesis. Endocrine Reviews, 1996; 17(5), 481-517.
46. Trujillo AI, Casal A, Penagaricano F, et al. Association of SNP of neuropeptide Y, leptin, and IGF-1 genes with residual feed intake in confinement and under grazing condition in Angus cattle. Journal of Animal Science, 2013; 91(9), 4235-4244.
47. Dar MR, Singh M, Thakur S, et al. Exploring the relationship between polymorphisms of leptin and IGF-1 genes with milk yield in indicine and taurine crossbred cows. Tropical Animal Health and Production, 2021; 53(4), 413.
48. Montelli NLLL, Alvarenga TIRC, Almeida AK., et al. Associations of feed efficiency with circulating IGF-1 and leptin, carcass traits and meat quality of lambs. Meat Science, 2021; 173, 108379.
49. Darwish AM, Abdelhafez MA, Abdel-Hamid ZG, et al. Correlation analysis between polymorphism of leptin and IGFI genes and body measurements in Barki and Farafra sheep. Beni-Suef University Journal of Basic and Applied Sciences, 2023; 12(1), 119.
50. Morissette MR, Cook SA, Buranasombati C, et al. Myostatin inhibits IGF-I-induced myotube hypertrophy through Akt. American Journal of Physiology-Cell Physiology, 2009; 297, C1124–C1132.
51. Ardicli S, Dincel D, Samli H, et al. Association of polymorphisms in lipid and energy metabolism-related genes with fattening performance in Simmental cattle. Animal Biotechnology, 2023; 34(8), 3428-3440.
52. Bongiorni S, Mancini G, Chillemi G, et al. Identification of a short region on chromosome 6 affecting direct calving ease in Piedmontese cattle breed. PLoS One, 2012; 7(12), e50137.
53. Carvalho TDD, Siqueira F, Torres Júnior RADA, et al. Association of polymorphisms in the leptin and thyroglobulin genes with meat quality and carcass traits in beef cattle. Revista Brasileira de Zootecnia, 2012; 41, 2162-2168.
54. Woronuk GN, Marquess FL, James ST, et al. Association of leptin genotypes with beef cattle characteristics. Animal Genetics, 2012; 43(5), 608-610.
55. Sedykh TA, Kalashnikova LA, Gizatullin RS et al. Effects of leptin gene polymorphism on beef cattle performance. Russian Agricultural Sciences, 2020; 46(6), 614-618.
56. Matsumoto H, Kimura S, Nagai Y, et al. Leptin gene contributes to beef marbling standard, meat brightness, meat firmness, and beef fat standard of the Kumamoto sub-breed of Japanese Brown cattle. Animal Science Journal, 2022; 93(1), e13698.
57. Couto CE, Livramento KGD, Paiva LV, et al. DGAT and LEP gene polymorphisms and their association with carcass characteristics and the lipid profile of meat from Nellore cattle. Revista Brasileira de Saúde e Produção Animal, 2023; 24, 20220012.
58. Valencia CPL, Franco LÁÁ, Herrera DH. Association of single nucleotide polymorphisms in the CAPN, CAST, LEP, GH, and IGF-1 genes with growth parameters and ultrasound characteristics of the Longissimus dorsi muscle in Colombian hair sheep. Tropical Animal Health and Production, 2022; 54(1), 82.
59. Liu Y, Kong L, Li S, et al. Correlation and regression analysis of GH and IGF-1 genes in Liaoning cashmere goats with body size and other production performance. Journal of Genetic Engineering and Biotechnology, 2024; 22(4), 100440.
60. Kader Esen V, Esen S. Association of the IGF1 5'UTR polymorphism in meat-type sheep breeds considering growth, body size, slaughter, and meat quality traits in Turkey. Veterinary Sciences, 2023; 10(4), 270.
61. Meira AN, Montenegro H, Coutinho L., et al. Single nucleotide polymorphisms in the growth hormone and IGF type-1 (IGF1) genes associated with carcass traits in Santa Ines sheep. Animal, 2019; 13(3), 460-468.
62. Islam KK, Vinsky M, Crews RE, et al. Association analyses of a SNP in the promoter of IGF1 with fat deposition and carcass merit traits in hybrid, Angus and Charolais beef cattle. Animal Genetics, 2009; 40, 766–769.
63. Pereira AGT, Utsunomiya YT, Milanesi M, et al. Pleiotropic genes affecting carcass traits in Bos indicus (Nellore) cattle are modulators of growth. PLoS One, 2016; 11(7), e0158165.
64. Cobanoglu O, Ardicli S.Genetic variation at the OLR1, ANXA9, MYF5, LTF, IGF1, LGB, CSN3, PIT1, MBL1, CACNA2D1, and ABCG2 loci in Turkish Grey Steppe, Anatolian Black, and East Anatolian Red cattle. Turkish Journal of Veterinary & Animal Sciences, 2022; 46(3), 494-504.
65. Gerasimov NP, Dzhulamanov KM, Lebedev SV, et al. Effect of IGF-1 C472T, GH C2141G, and GHR T914A polymorphisms on growth performance and feed efficiency in young Kazakh white-headed cattle. Veterinary World, 2023; 16(8), 1584.
66. Saleh AA, Hassan TG, El-Hedainy DK, et al. IGF-I and GH Genes polymorphism and their association with milk yields, composition and reproductive per-

formance in Holstein–Friesian dairy cattle. BMC Veterinary Research, 2023; 20(1), 341.
67. Sen U, Gokcek, D, Yılmaz OF, et al. Relationships among growth, carcass characteristics, and Myf5, Myf6, MyoD, and MyoG genes expression level in Saanen male kids with varying slaughter weights. Animals, 2024; 15(1), 16.
68. Daldaban F, Arslan K, Aksel EG, et al. Polymorphism of the STAT5A and MYF-5 genes in Anatolian water buffalo. Turkish Journal of Veterinary & Animal Sciences, 2020; 44(2), 284-289.
69. Zayed MA, El-Kholy AF, Shehata MF, et al. Genetic contribution of myogenic factor 5 and growth hormone genes for live body measurements, carcass traits and meat quality of dromedary camel. Journal of Animal and Poultry Production, 2022; 13(10), 153-156.
70. Fadhil M, Zülkadir U. Association between polymorphisms of Myf5, MSTN and CAST genes and fattening performance in Brown Swiss and Holstein cattle breeds. Animal Biotechnology, 2021; 32(1), 121-129.
71. Ardicli S, Ustuner H, Arslan O, et al. Variability of CAPN1 g. 5709 C> G and MYF5 g. 1911 A> G Polymorphisms in Beef Cattle Imported from Brazil to Turkey. Lalahan Hayvancılık Araştırma Enstitüsü Dergisi, 2019b; 59(2), 72-78.
72. MacNeil MD, Nkrumah JD, Woodward BW, et al. Genetic evaluation of Angus cattle for carcass marbling using ultrasound and genomic indicators. Journal of Animal Science, 2010; 88(2), 517-522.
73. Ardicli S. Genetik ve postmortem mekanizmaların sığır eti renk özellikleri üzerine etkisi. Uludağ Üniversitesi Veteriner Fakültesi Dergisi, 2018; 37(1), 49-59. https://doi.org/10.30782/uluvfd.398947
74. Ustuner H, Ardicli S, Arslan O, Brav FC. Fattening performance and carcass traits of imported Simmental bulls at different initial fattening age. Large Animal Review, 2020; 26: 161-165.
75. Ustuner H, Ardicli S, Arslan O. Determination of the alterations in quality parameters and consumer preference of dry-aged beef based on different periods of ageing using a purposive incubator. Journal of the Hellenic Veterinary Medical Society, 2021; 72(1): 2669-2676. https://doi.org/10.12681/jhvms.26749
76. Ardicli S, Ardicli O, Ustuner H. Unraveling the Complexities of Beef Marination: Effect of Marinating Time, Marination Treatments, and Breed. Foods, 2024, 13(18): 2979. https://doi.org/10.3390/foods13182979
77. Ardicli, S. Analysis of IGF1 C472T and TG C422T polymorphisms in Turkish Grey Steppe and Holstein crossbred bulls. 2018. Uludağ Üniversitesi Veteriner Fakültesi Dergisi, 37(1), 21-28.
78. Ardicli, S., Aldevir, O., Aksu, E., & Gumen, A. The variation in the beta-casein genotypes and its effect on milk yield and genomic values in Holstein-Friesian cows. 2023. Animal Biotechnology, 34(8), 4116-4125.
79. Ardicli, S., Aldevir, Ö., Aksu, E., Kucuk, K., & Gümen, A. Associations of bovine beta-casein and kappa-casein genotypes with genomic merit in Holstein Friesian cattle. 2024. Archives Animal Breeding, 67(1), 61-71.
80. Ardicli, S., Senturk, N., Bozkurt, B., Babayev, H., Selvi, T., Skolnick, S., ... & Cobanoglu, O. The impact of genetic variants related to the fatty acid metabolic process pathway on milk production traits in Jersey cows. 2024. Animal Biotechnology, 35(1), 2396421.
81. Saleh, A.A., Hassan, T.G.M., EL-Hedainy, D.K.A. et al. IGF-I and GH Genes polymorphism and their association with milk yields, composition and reproductive performance in Holstein–Friesian dairy cattle. BMC Vet Res 20, 341 (2024). https://doi.org/10.1186/s12917-024-04188-4.