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Individual and combined effects of *CAPN1*, *CAST*, *LEP* and *GHR* gene polymorphisms on carcass characteristics and meat quality in Holstein bulls

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Abstract. The objective of this study was to determine the association of single nucleotide polymorphisms (SNPs) with carcass characteristics and meat quality traits in selected candidate genes in Holstein bulls. Five SNPs in four genes, i.e. calpain 1 (*CAPN1*), calpastatin (*CAST*), leptin (*LEP*) and growth hormone receptor (*GHR*), were genotyped in 400 purebred bulls using PCR-RFLP. Statistically significant associations were as follows: *CAPN1* G316A with live weight, carcass weight, back fat thickness, m. longissimus thoracis et lumborum area and carcass measurements; *CAPN1* V530I with pH and L^*; *CAST* S20T with live weight, inner chest depth and b^* value; and *GHR* with ph, a^* and h^*. In addition, significant genotypic interactions were observed for dressing percentage (*LEP* A80V × *CAST* S20T), pH (*CAPN1* V530I × *GHR* S555G and *LEP* A80V × *GHR* S555G) and rump width (*CAPN1* V530I × *CAST* S20T). There was no association between the *LEP* A80V marker and any of the traits evaluated, nor was there any association of the tested SNPs with chest width, C^* and marbling score. The present results could therefore be indicative for future studies on meat yield and quality.

1 Introduction

Selection for increased meat production and quality in the beef industry have been the primary emphasis of selection programmes. Recently, the trend of improving these programmes has gradually changed from traditional phenotypical selection methods to genotypic selection by utilizing molecular markers and a better understanding of DNA polymorphisms that have an effect on carcass and meat quality may lead to important applications through marker-assisted selection (MAS) programmes (Guo et al., 2016). However, genetic variations regarding both the quantity and quality of beef already exist among breeds and even among different populations of the same breed (Burrow et al., 2001). In 2016, 1.2 million tonnes of red meat was produced from 9.8 million animals slaughtered in Turkey. The slaughter population was made up of 3.9 million cattle, 4.1 million sheep, 1.8 million goats and 1400 water buffaloes. Of this production, 1.1 million tonnes (approximately 91 % of total) was composed of beef (Turkish Statistical Institute, 2016). In some countries, the beef industry is exclusively based on specific beef herds, but conversely, cattle farms in many countries, such as Turkey, generally consist of dairy cattle and dual-purpose breeds, and the number of beef breeds is limited. Among these, the Holstein breed comprises by far the most common cattle breed in Turkey, with 5.5 millions purebreds and 856 000 crossbreeds. Considering the approximately 14 million total cattle count, the Holstein breed has a significant impact on Turkish animal husbandry (Turkish Ministry of Food, Agriculture and Livestock Database, 2015). Therefore, evaluating the potential of Holstein meat can be considered as a strategically important point in breeding programmes and meat production (Ardicli et al., 2017a).

In recent years, genes associated with meat quality have been identified and single nucleotide polymorphisms (SNPs) of many candidate genes have been specifically determined (Li et al., 2013). In this context, one of the most investigated genes is bovine micromolar calcium-activated neutral protease 1 (*CAPN1*), which is located on chromosome 29, encoding the large subunit of the enzyme μ-calpain involved

in degrading myofibrillar proteins under postmortem conditions. *CAPN1* was suggested as a genetic factor influencing the postmortem tenderization process (Koohmaraie, 1996; Page et al., 2002; Miquel et al., 2009). The SNP G316A in exon 9 (alleles C/G) of the *CAPN1* gene has been associated with meat quality traits (Gill et al., 2009; Miquel et al., 2009; Smith et al., 2009; Bonilla et al., 2010; Mazzucco et al., 2010; Pinto et al., 2010; Kaneda et al., 2011), final weight and average daily weight gain (Miquel et al., 2009; Tait et al., 2014). The SNP V530I in exon 14 of the *CAPN1* gene has been associated with beef tenderness (Corva et al., 2007; Allais et al., 2011), meat colour and drip loss (Ribeca et al., 2013). The bovine calpastatin (*CAST*) gene, mapped to chromosome 7, is considered a candidate gene for beef tenderness (Schenkel et al., 2006). The S20T polymorphism in the *CAST* gene has been shown to be associated with meat quality traits (Juszczuk-Kubiak et al., 2004). The leptin (*LEP*) gene, also known as the "obese gene", located on bovine chromosome 4, encodes leptin, which is secreted by adipose tissue. The concentration of leptin can be involved in food consumption, energy expenditure and adipose tissue development (Buchanan et al., 2002; Lagonigro et al., 2003) and plays an indicator role in marbling, intramuscular fat content, back fat depth and meat quality grade in feedlot cattle (Geary et al., 2003; Li et al., 2013). The A80V polymorphism in exon 3 has been shown to be a candidate marker for milk yield and composition traits (Liefers et al., 2003; Kulig, 2005; Kulig et al., 2010); however, its association with meat quality traits has not been fully depicted. Growth hormone (GH), also known as somatotropin, plays an important role in growth and metabolism (Di Stasio et al., 2005; Waters et al., 2011) by interacting with a specific receptor (GHR), which activates an intracellular signalling pathway (Zhou and Jiang, 2006). Variations in the *GHR* gene, mapped to chromosome 20, have been associated with performance traits in cattle (Blott et al., 2003; Ge et al., 2003; Viitala et al., 2006; Garrett et al., 2008; Waters et al., 2011). The polymorphism in exon 10 of the *GHR* gene has been associated with meat quality (Di Stasio et al., 2005; Reardon et al., 2010), growth performance and body size traits (Waters et al., 2011).

The genetic markers studied in our study and their association with meat yield/quality have also been investigated by other researchers, but the results were often inconsistent. In addition, there is very limited information about the effects of these markers on meat yield and quality of the Holstein breed, which constitutes a significant proportion in Turkey's meat industry. Therefore, the aim of this study was to investigate associations of SNPs at *CAPN1*, *CAST*, *LEP* and *GHR* genes with carcass characteristics, meat yield and quality traits.

2 Materials and methods

2.1 Animals, management and slaughter procedures

Data from 400 Holstein bulls randomly selected from a commercial herd, with a herd size 20 000 cattle, located in South Marmara region and slaughtered at 14–21 months of age, were used in the study. All animals were recorded for the Pedigree Project of the Turkish Ministry of Food, Agriculture and Livestock, and Cattle Breeders Association. Ethical approval was received from Uludag University (approval number: 2012-10/05). The farm was located in Bandırma, northern Balıkesir province (40°18′06.0″ N and 27°56′28.5″ E). The animals were maintained in a semi-open free-stall barn, with straw as bedding. Maximum and minimum ambient air temperatures (°C) in the sheds during the period of the study were 10.1 ± 1.1 and 2.1 ± 0.4 in winter, 19.1 ± 1.4 and 7.06 ± 1.4 in spring and 30.5 ± 1.9 and 16.8 ± 0.8 in summer and relative humidity percentages (%) were 66.7 ± 1.4, 58.9 ± 2.4 and 70.5 ± 0.8 in the same seasons, respectively. The fattening period were initiated after 2 weeks of training. During the fattening period, growing and finishing rations contained corn, potato and tomato pomace silage; barley straw; barley butter; pasta; corn; corn gluten meal; corn bran; sugar-beet pulp; soybean meal; sunflower meal; vitamin and mineral premix; limestone; and salt. The growing ration contained 13.8 % of crude protein and 10.2 MJ kg^{-1} of metabolizable energy on a dry matter basis and the finishing ration contained 10.3 % of crude protein and 11.5 MJ kg^{-1} of energy on a dry matter basis. All animals were fed ad libitum with the same diets and had full access to water throughout the experiment. At the end of the finishing period, the animals, in a non-fasted state, were transported to the nearest slaughterhouse (40°21′23.6″ N and 27°56′41.1″ E). The duration of transport from farm to slaughterhouse was approximately 1–2 h. Prior to slaughter, final live weight (LW) was recorded by precision scale (100 g sensitivity). Cattle were stunned by captive bolt before being slaughtered by means of exsanguination and dressed using standard commercial practices, after being kept for 24 h in paddocks and deprived of feed but with full access to water. After slaughter, all of the carcasses were electrically stimulated for a duration of 30 s (60 V), suspended through the Achilles tendons and chilled for 24 h at 4 °C.

2.2 Carcass characteristics

In the present study, non-carcass components were removed and then hot carcass weight (HCW) was determined. Hot carcass did not include kidneys and perinephric or pelvic fat. Chilled carcass weight (CCW) was measured after 24 h at 4 °C and the dressing percentage (DP) was calculated based on HCW. After slaughter, carcass measurements including carcass length (CL), rump length (RL), rump width (RW), chest width (CW) and inner chest depth (ICD) were mea-

sured with a caliper, cane and ruler according to following anatomic regions as described by Sagsoz et al. (2005): CL – the distance from the os pubis to the tip of the first rib; RL – the distance from the os calcaneus to the median point of the os pubis; RW – from the rump circumference starting from the point opposite the meat section to the line connecting the centre of the os pubis and the os calcaneus; CW – outer side of the half carcass section from the sixth rib tip to the sixth vertebra; ICD – measured from the sixth rib tip to the sixth vertebra on the inner side of the half carcass section. Back fat thickness (BFT) was measured from the lateral side of the m. longissimus thoracis et lumborum (LTL) at the 12th rib and the same rib surface was evaluated to calculate the LTL area by using a planimeter (Ushikata X-Plan 380d III, Tokyo, Japan).

2.3 Meat quality analyses

Meat quality characteristics investigated in the current study were marbling score (MS), carcass pH and meat colour (L^*, a^*, b^*, C^* and h^*). Marbling was subjectively evaluated corresponding to fat distribution among the muscle fibres in the m. longissimus thoracis (LT) at the 12th–13th rib interface to represent 9 ° (practically devoid, traces, slight, small, modest, moderate, slightly abundant, moderately abundant, abundant) of marbling (Hilton et al., 1998). Carcass pH was measured in the LT between the 12th and 13th ribs at 24 h postmortem using a digital pH meter (Testo 205, Lenzkirch, Germany). Meat colour parameters including L^* (lightness), a^* (redness) and b^* (yellowness) values were evaluated using a spectrocolorimeter (Konica Minolta CM508d, Minolta Co., Ltd, Osaka, Japan) with illuminant D65 as the light source. The device was set to make three measurements and take their average after the calibration corresponding to the standard white plate. Three-times-repeated colour measurements were performed from each sample of the LTL after 24 h storage at 4 °C on cut surface of fat-free area and the average of these measurements was evaluated as the final value (Ekiz et al., 2009). Chroma value (C^*) was calculated as $(a^{*2} + b^{*2})^{1/2}$ and hue angle (h^*) as arctan (b^*/a^*).

2.4 Genomic DNA isolation

DNA was isolated from 4 mL blood samples obtained from the vena jugularis of each bull and collected in K_3EDTA tubes (Vacutest Kima, SRL, Italy) by a phenol–chloroform method as described by Green and Sambrook (2012). The amount and purity of the DNA samples was measured with a spectrophotometer (NanoDrop 2000c, Thermo Scientific, Wilmington, DE, USA). DNA samples were stored at −80 °C until the genotyping was performed.

2.5 Markers used and genotyping

In the present study, the polymorphisms in four candidate genes were genotyped, which included the G316A and the V530I in the *CAPN1* gene, the S20T in the *CAST* gene, A80V in the *LEP* gene and the S555G in the *GHR* gene. Marker G316A (GenBank accession number: AF252504) is a cytosine/guanine (C/G) polymorphism in exon 9 of the *CAPN1* gene that produces an amino acid substitution (glycine/alanine) in position 316. Marker V530I (GenBank accession number: AF248054) of the same gene is an adenine/guanine (A/G) polymorphism in exon 14 that also produces an amino acid substitution (isoleucine/valine) in position 530 (Page et al., 2002; Casas et al., 2005). The SNP S20T (GenBank accession number: AF117813) in the *CAST* gene is a guanine/cytosine (G/C) polymorphism located in exon 1C/1D that produces serine/threonine substitution at protein position 20 (Juszczuk-Kubiak et al., 2004). Marker A80V (GenBank accession number: AF536174.1) in exon 3 of the *LEP* gene expresses the existence of a cytosine/thymine (C/T) substitution that causes coding of alanine instead of valine in position 80 (Haegeman et al., 2000; Lagonigro et al., 2003). Marker S555G (GenBank accession number: AF140284) is the guanine/adenine (G/A) polymorphism at position 257 in exon 10 and induces serine/glycine substitution in position 555 of the *GHR* gene (Di Stasio et al., 2005).

Genotyping of the SNPs in the *CAPN1*, *CAST*, *LEP* and *GHR* genes was performed by PCR-RFLP. Primer sequences and PCR conditions for amplification are shown in Table 1. The PCR amplification was performed in a total volume of 50 µL containing 33.5 µL of ddH_2O, 5 µL of 10× buffer, 5 µL of $MgSO_4$, 1 µL of dNTPs (2.5 mM), 2.5 U of Taq DNA polymerase (Biomatik, Cambridge, Canada, A1003-500U, 5U µL^{-1}), 1 µL (0.025 µM) of each primer, and 3 µL of the DNA sample at a concentration of 100 ng µL^{-1}. The DNA amplification reactions were performed in a thermal cycler (Palm Cycler GC1-96, Corbett Research, Australia). After amplification, 15 µL of the PCR product with each SNP was digested in 15 U of the corresponding restriction enzyme (Table 1) by incubating at 37 °C for 16 h. Afterwards, the digestion products were electrophoresed in 3 % agarose gel (Sigma Aldrich, Steinheim, Germany) at 85–90 V for 1 h after incubation and visualized by a gel imaging system (DNr-Minilumi, DNR Bio-Imaging Systems, Israel).

2.6 Statistical analysis

The Hardy–Weinberg equilibrium (HWE) was tested for all alleles by using POPGENE software v1.32 (Yeh et al., 2000). The population genetic indexes including gene heterozygosity (He), effective allele numbers (Ne) and polymorphism information content (PIC) were estimated as described by Nei and Roychoudhury (1974) and Botstein et al. (1980). Association analysis was carried out by the least-squares method

Table 1. Primers sequences (from 5′ to 3′), PCR conditions and restriction enzymes used for genotyping the polymorphisms in the current study.

SNP name*	Allele	PCR amplicon (bp)	Primers (5′ to 3′)	PCR conditions	Restriction enzyme	Reference
CAPN1 G316A			F: 5′GACTGGGGTCTCTGGACTT3′ R: 5′GGAACCTCTGGCTCTTGA3′	95 °C 5′ (95 °C 45 s, 63 °C 45 s, 72 °C 45 s) 35 cycles, 72 °C 5′	*Btg*I	Lisa and Di Stasio (2009)
CAPN1 V530I	A/G	787	F: 5′AGCGCAGGGACCCAGTGA3′ F: 5′TCCCCTGCCAGTTGTCTGAAG3′	95 °C 5′ (95 °C 1′, 63 °C 1′, 72 °C 1′) 35 cycles, 72 °C 5′	*Ava*II	Soria et al. (2010)
CAST S20T	G/C	624	F: 5′TGGGGCCCAATGACGCCATCGATG3′ R: 5′GGTGGAGCAGCACTTCTGATCACC3′	94 °C 5′ (94 °C 30 s, 62 °C 45 s, 72 °C 45 s) 32 cycles, 72 °C 5′	*Alu*I	Juszczuk-Kubiak et al. (2004)
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	Oztabak et al. (2010)
GHR S555G	G/A	342	F: 5′GCTAACTTCATCGTGGACAAC3′ R: 5′CTATGGCATGATTTTGTTCAG3′	95 °C 5′ (94 °C 45 s, 53 °C 30 s, 72 °C 50 s) 35 cycles, 72 °C 5′	*Alu*I	Di Stasio et al. (2005)

CAPN1 – micromolar calcium-activated neutral protease 1. *CAST* – calpastatin. *LEP* – leptin. *GHR* – growth hormone receptor. * SNP names were used according to translation.

as applied in a general linear model (GLM) procedure of Minitab (MINITAB®, Pennsylvania, USA, v17.1.0) according to the following statistical model:

$$Y_{ijklmnop} = \mu + S_i + W_j + \mathrm{AG}_k + \mathrm{BG}_l + \mathrm{CG}_m + \mathrm{DG}_n + \mathrm{EG}_o + e_{ijklmnop},$$

where $Y_{ijklmnop}$ represents the studied traits, μ the overall mean, S_i the fixed effect of season at the slaughter (i = winter, spring and summer), W_j the fixed effect of age at slaughter (j = 14–21 months), AG_k the fixed effect of the *CAPN1* genotype for the G316A (k = CC, CG, GG), BG_l the fixed effect of the *CAPN1* genotype for the V530I (l = AA, AG, GG), CG_m the fixed effect of the *CAST* genotype for the S20T (m = CC, CG, GG), DG_n the fixed effect of the *LEP* genotype for the A80V (n = CC, CT, TT), EG_o the fixed effect of the *GHR* genotype for the S555G (o = AA, AG, GG) and $e_{ijklmnop}$ the random residual effect.

The models in the present study were selected by evaluating the adjusted R^2 to compare the explanatory power of models with different numbers of predictors. Markers were initially evaluated using the significance of genotype effects for each trait. Afterwards, the interactions between *CAPN1*, *CAST*, *LEP* and *GHR* genotypes were added to the model and tested for significance. When significant associations were identified, the mean values for each genotype were contrasted using Tukey's test.

3 Results

3.1 Allele, genotype frequencies and population genetic indices

Two alleles and three genotypes in each SNP were found in the present study. The gene frequencies, population genetic indices including heterozygosity (He), effective allele numbers (Ne), polymorphism information content (PIC) and compatibility with the Hardy–Weinberg equilibrium (HWE) are shown in Table 2. A total of 400 individuals were used in the study. HWE was tested for all alleles and genotypic frequencies and was compatible except for the *LEP* A80V and *GHR* S555G polymorphisms in Holstein population. Results indicated that the genotypes CC and AA, at the G316A and V530I markers of the *CAPN1* gene, respectively, had relatively low frequencies but an adequate number of animals had these genotypes to estimate their association with phenotypic traits. The genotype frequency of GC (52 %) was rather high compared to the other two genotypes for the *CAST* S20T marker. In addition, the genotypic frequencies of TT (65 %) and AA (63.5 %) at the *LEP* A80V and *GHR* S555G markers, respectively, were remarkably high in the current study. The minor allele frequencies (MAF) ranged from 0.18 to 0.43, while Ne ranged from 1.42 to 1.96. Nevertheless, the *CAPN1* V530I marker showed the low frequency of the allele A (0.18), resulting in low genetic variabilities of He (0.2952) and PIC (0.2516) compared with other SNPs showing relatively high values of He (ranged from 0.3648 to 0.4902) and PIC (ranged from 0.2982 to 0.3705).

3.2 Marker associations

Levels of significance, least-squares means, and standard errors are reported in Tables 3 and 4 for the effects of *CAPN1* G316A and V530I, *CAST* S20T, *LEP* A80V and *GHR* S555G on carcass characteristics and meat quality. The marker *CAPN1* G316A was highly associated with LW, HCW, CCW and LTL area ($P < 0.001$). In addition, the same marker at *CAPN1* gene affected the BFT ($P < 0.01$). Moreover, *CAPN1* G316A marker was also associated with CL, RL, RW and ICD at different levels of significance (Table 4). The results indicated that the GG genotype had noteworthy effects on the mentioned carcass measurement, supporting the results of evaluating live weight and carcass weights. The SNP V530I of the *CAPN1* gene had a significant effect on pH at 24 h ($P < 0.05$). Animals with the AA genotype had higher values for pH of LT compared to the other two genotypes (Table 3). In addition, AA genotype was significantly associated with L^* value. Correspondingly, meat colour eval-

Table 2. Gene frequencies, population genetic indices and HWE test of polymorphisms in the *CAPN1*, *CAST*, *LEP* and *GHR* genes in a Holstein population.

Gene	*CAPN1*							*CAST*			*LEP*			*GHR*	
SNP	G316A				V530I			S20T			A80V			S555G	
Genotypes	CC	CG	GG	AA	AG	GG	GG	GC	CC	CC	CT	TT	AA	GA	GG
N	26	169	205	12	119	269	67	208	125	59	81	260	254	100	46
%	6.50	42.25	51.25	3.00	29.75	67.25	16.75	52.00	31.25	14.75	20.25	65.00	63.50	25.00	11.50
MAF		0.28			0.18			0.43			0.25			0.24	
He		0.4032			0.2952			0.4902			0.3750			0.3648	
Ne		1.68			1.42			1.96			1.60			1.57	
PIC		0.3219			0.2516			0.3705			0.3046			0.2982	
$\chi 2$ (HWE)		1.28			0.07			1.55			83.97*			39.61*	

χ^2 (HWE) – Hardy–Weinberg equilibrium. χ^2 value – *N* – number of experimental bulls. MAF – minor allele frequency. He – heterozygosity. Ne – number of effective alleles. PIC – polymorphism information content. * $P < 0.001$, not consistent with HWE.

uation indicated that meat from GG animals had a higher L^* value.

The *CAST* S20T marker was effective on LW, ICD and b^* value ($P < 0.05$). Animals with the GG genotype had significantly higher values of LW and ICD but a lower b^* value compared to the other two genotypes (Tables 3 and 4). No association was observed between the *LEP* A80V and any of the phenotypic traits evaluated. The marker *GHR* S555G showed associations with pH at 24 h, a^* and h^*. Higher pH and lower a^* were observed in meat from individuals with GG genotype than those carrying the AA and GA genotypes. There was no association between any of the tested SNPs with DP (individual effects of the markers) and CW traits, nor was there any association with variation in C^* and MS ($P > 0.05$).

In this study, the *CAST* S20T × *LEP* A80V interaction was associated with DP ($P < 0.05$); the *CAPN1* V530I × *GHR* S555G and the *LEP* A80V × *GHR* S555G interactions were associated with variation in pH values ($P < 0.01$) and the *CAST* S20T × *CAPN1* V530I interaction was associated with RW ($P < 0.01$), as shown in Table 5.

4 Discussion

The primary objective of the current study was to determine whether DNA markers commonly studied (*CAPN1* G316A and V530I, *CAST* S20T, *LEP* A80V and *GHR* S555G) in various beef cattle populations could be applied in Holstein bulls, which comprise by far the most important share of meat production in Turkey. The present results showed a deviation from HWE for the *LEP* A80V and *GHR* S555G polymorphisms in Holstein population. Deviations from HWE at particular markers may be associated with population characteristics. Accordingly, this disequilibrium can be a result of inbreeding or indirect selection for these loci from the selection for milk production in the Holstein breed (Lacorte et al., 2006). Menezes et al. (2006) described a polymorphic locus as the frequency of the most common allele being lower than 0.95; accordingly all markers used in the present study were polymorphic. Further, these markers are considered as mildly informative according to the classification reported by Botstein et al. (1980). *CAPN1*, *CAST*, *LEP* and *GHR* genes were chosen because they have been shown to be involved in the regulation of appetite, growth rate, carcass traits and meat quality in many beef cattle breeds, and our results indicated that *CAPN1*, *CAST* and *GHR* markers may be associated with carcass traits and meat quality. Among them, *CAPN1* G316A was highly associated with LW and carcass weights ($P < 0.001$) given that the G is the favourable allele for these traits. Animals with the GG genotype had +52.7 kg heavier LW and +34.4 kg heavier HCW compared to the CC genotype in the present study. In the literature, *CAPN1* G316A was evaluated as an effective marker on beef tenderness in several studies indicating that the C allele is associated with more tender meat (Casas et al., 2005; Corva et al., 2007; Gill et al., 2009; Miquel et al., 2009; Smith et al., 2009; Curi et al., 2010). It has been shown that, along with loci affecting beef tenderness, other loci associated with weaning weight and carcass weights were mapped to bovine chromosome 29 (Casas et al., 2005). Miquel et al. (2009) reported that final weight and average daily gain differentiated between the *CAPN1* G316A marker genotypes and that choosing animals with the favourable marker genotype (CC) for tenderness resulted in a selection of animals with lower average daily gain and final weight in Angus and Brangus steers. In addition, Pintos and Corva (2011) found significant associations between the same marker with birth weight, weaning weight and live weight recorded at 18 months of age in Angus cattle. Ardicli et al. (2017b) reported that homozygous animals for allele G at the *CAPN1* G316A marker reached the highest final weight and total weight gain in a shorter fattening period with higher average daily gain in Simmental bulls. Among the factors considered, it is possible that Warner–Bratzler shear force (WBSF) values show an association with LW and that selection for this marker may lead to changes in both traits. Conversely, Corva et al. (2007) and Tait et al. (2014) reported that final body weight and carcass weight were not affected by the *CAPN1* G316A marker genotypes. The breed

Table 3. Levels of significance, least-squares means, and standard errors for the effect of *CAPN1*, *CAST*, *LEP* and *GHR* on carcass traits and meat quality in a Holstein population.

Genotype	*N*	LW (kg)	HCW (kg)	CCW (kg)	DP (%)	BFT (mm)	LTLA (cm^2)	MS (1–9)	pH
CAPN1 G316A									
CC	26	437.8 ± 12.07^{b}	229.6 ± 6.83^{b}	225.6 ± 6.75^{b}	53.36 ± 0.47	2.27 ± 0.22^{b}	90.89 ± 3.01^{b}	2.76 ± 0.22	5.70 ± 0.04
CG	169	481.5 ± 7.61^{a}	259.0 ± 4.30^{a}	254.7 ± 4.26^{a}	53.54 ± 0.32	2.81 ± 0.14^{a}	101.92 ± 1.92^{a}	2.70 ± 0.13	5.70 ± 0.03
GG	205	493.5 ± 7.64^{a}	264.0 ± 4.32^{a}	259.7 ± 4.28^{a}	53.48 ± 0.30	2.98 ± 0.14^{a}	102.47 ± 1.94^{a}	2.61 ± 0.13	5.71 ± 0.03
		$P < 0.001$	$P < 0.001$	$P < 0.001$	NS	$P < 0.01$	$P < 0.001$	NS	NS
CAPN1 V530I									
AA	12	464.9 ± 15.80	248.0 ± 8.91	243.8 ± 8.84	53.06 ± 0.63	2.57 ± 0.29	100.98 ± 4.08	2.59 ± 0.28	5.89 ± 0.07^{a}
AG	119	475.3 ± 7.15	252.7 ± 4.07	248.5 ± 4.00	53.06 ± 0.28	2.74 ± 0.13	97.92 ± 1.79	2.71 ± 0.12	5.63 ± 0.02^{b}
GG	269	472.6 ± 6.58	251.9 ± 3.70	247.7 ± 3.68	53.21 ± 0.26	2.74 ± 0.12	96.37 ± 1.64	2.76 ± 0.11	5.59 ± 0.02^{b}
		NS	NS	NS	NS	NS	NS	NS	$P < 0.05$
CAST S20T									
GG	67	482.9 ± 9.54^{a}	256.4 ± 5.40	252.2 ± 5.34	52.87 ± 0.38	2.77 ± 0.17	97.83 ± 2.41	2.75 ± 0.16	5.71 ± 0.03
GC	208	464.7 ± 7.97^{b}	247.3 ± 4.51	243.1 ± 4.46	53.09 ± 0.31	2.66 ± 0.14	98.08 ± 2.02	2.66 ± 0.13	5.70 ± 0.02
CC	125	465.2 ± 8.16^{b}	249.0 ± 4.62	244.7 ± 4.57	53.37 ± 0.32	2.63 ± 0.15	99.36 ± 2.06	2.66 ± 0.14	5.69 ± 0.02
		$P < 0.05$	NS	NS	NS	NS	NS	NS	NS
LEP A80V									
CC	59	471.8 ± 9.63	251.8 ± 5.45	247.6 ± 5.39	53.17 ± 0.38	2.63 ± 0.17	97.61 ± 2.42	2.66 ± 0.16	5.66 ± 0.03
CT	81	472.0 ± 8.92	251.3 ± 5.05	247.1 ± 4.99	53.06 ± 0.35	2.74 ± 0.16	98.56 ± 2.27	2.80 ± 0.15	5.73 ± 0.03
TT	260	469.0 ± 8.06	249.6 ± 4.56	245.3 ± 4.51	53.11 ± 0.32	2.68 ± 0.14	99.10 ± 2.02	2.60 ± 0.14	5.72 ± 0.02
		NS	NS	NS	NS	NS	NS	NS	NS
GHR S555G									
AA	254	473.9 ± 7.48	252.5 ± 4.23	248.3 ± 4.18	53.18 ± 0.29	2.62 ± 0.13	98.06 ± 1.89	2.68 ± 0.13	5.57 ± 0.02^{b}
GA	100	461.9 ± 8.59	246.4 ± 4.86	242.2 ± 4.81	53.19 ± 0.34	2.74 ± 0.15	99.94 ± 2.16	2.77 ± 0.14	5.58 ± 0.02^{b}
GG	46	477.0 ± 10.40	253.7 ± 5.88	249.4 ± 5.82	52.98 ± 0.41	2.70 ± 0.19	97.27 ± 2.60	2.62 ± 0.17	5.97 ± 0.03^{a}
		NS	NS	NS	NS	NS	NS	NS	$P < 0.01$

N – number of experimental bulls. LW – live weight. HCW – hot carcass weight. CCW – chilled carcass weight. DP – dressing percentage. BFT – back fat thickness. LTLA – m. longissimus thoracis et lumborum area. MS – marbling score. NS – not significant. [a,b] Different superscripts within a column indicate significant difference.

Table 4. Levels of significance, least-squares means, and standard errors for the effect of *CAPN1*, *CAST*, *LEP* and *GHR* on carcass measurement and meat colour in a Holstein population.

Genotype	*N*	CL (cm)	RL (cm)	RW (cm)	CW (cm)	ICD (cm)	L^*	a^*	b^*	C^*	h^*
CAPN1 G316A											
CC	26	138.7 ± 0.72^{c}	63.67 ± 0.42^{b}	98.16 ± 0.52^{b}	80.53 ± 0.75	58.58 ± 0.57^{b}	35.15 ± 0.94	10.55 ± 0.68	9.15 ± 0.66	14.27 ± 0.61	0.70 ± 0.04
CG	169	139.9 ± 0.43^{b}	63.83 ± 0.25^{b}	100.12 ± 0.31^{b}	81.26 ± 0.45	59.30 ± 0.34^{a}	33.69 ± 0.59	11.10 ± 0.43	9.14 ± 0.42	14.79 ± 0.38	0.67 ± 0.03
GG	205	140.9 ± 0.43^{a}	64.31 ± 0.25^{a}	100.89 ± 0.31^{a}	81.70 ± 0.45	59.86 ± 0.34^{a}	33.98 ± 0.60	10.69 ± 0.43	9.29 ± 0.42	14.6 ± 0.39	0.70 ± 0.03
		$P < 0.001$	$P < 0.05$	$P < 0.01$	NS	$P < 0.05$	NS	NS	NS	NS	NS
CAPN1 V530I											
AA	12	140.0 ± 0.92	64.11 ± 0.54	98.63 ± 0.68	81.49 ± 0.96	59.52 ± 0.73	32.47 ± 1.24^{b}	10.78 ± 0.89	9.18 ± 0.87	14.60 ± 0.80	0.70 ± 0.06
AG	119	139.6 ± 0.40	63.79 ± 0.23	100.33 ± 0.35	80.91 ± 0.41	59.03 ± 0.31	34.99 ± 0.56^{a}	10.89 ± 0.40	8.95 ± 0.39	14.46 ± 0.36	0.67 ± 0.02
GG	269	139.9 ± 0.37	63.91 ± 0.22	100.20 ± 0.29	81.09 ± 0.39	59.20 ± 0.29	35.37 ± 0.51^{a}	10.67 ± 0.37	9.45 ± 0.36	14.61 ± 0.33	0.70 ± 0.02
		NS	NS	NS	NS	NS	$P < 0.05$	NS	NS	NS	NS
CAST S20T											
GG	67	140.0 ± 0.53	64.10 ± 0.31	100.22 ± 0.38	81.57 ± 0.56	59.83 ± 0.42^{a}	34.14 ± 0.74	10.98 ± 0.54	$8.80^{b} \pm 0.52$	14.47 ± 0.48	0.66 ± 0.03
GC	208	139.8 ± 0.45	63.87 ± 0.26	100.02 ± 0.32	80.94 ± 0.47	58.96 ± 0.36^{b}	33.82 ± 0.62	10.76 ± 0.45	$9.70^{a} \pm 0.44$	14.88 ± 0.40	0.72 ± 0.03
CC	125	139.7 ± 0.46	63.84 ± 0.27	99.62 ± 0.33	80.98 ± 0.48	58.95 ± 0.37^{b}	34.87 ± 0.64	10.60 ± 0.46	$9.08^{a} \pm 0.45$	14.32 ± 0.41	0.69 ± 0.03
		NS	NS	NS	NS	$P < 0.05$	NS	NS	$P < 0.05$	NS	NS
LEP A80V											
CC	59	140.0 ± 0.54	64.28 ± 0.31	99.95 ± 0.38	81.19 ± 0.56	59.13 ± 0.42	34.15 ± 0.75	10.17 ± 0.54	8.86 ± 0.53	14.90 ± 0.49	0.69 ± 0.03
CT	81	139.5 ± 0.50	63.82 ± 0.29	99.79 ± 0.36	81.24 ± 0.52	59.26 ± 0.39	34.97 ± 0.70	11.32 ± 0.50	9.15 ± 0.49	14.94 ± 0.45	0.68 ± 0.04
TT	260	140.0 ± 0.46	63.71 ± 0.27	100.12 ± 0.33	81.07 ± 0.48	59.35 ± 0.36	33.70 ± 0.63	10.85 ± 0.45	9.58 ± 0.44	14.83 ± 0.41	0.71 ± 0.03
		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
GHR S555G											
AA	254	139.8 ± 0.43	63.76 ± 0.25	100.06 ± 0.31	81.38 ± 0.45	59.34 ± 0.34	34.50 ± 0.58	11.47 ± 0.42^{a}	8.71 ± 0.41	14.80 ± 0.38	0.64 ± 0.02^{b}
GA	100	139.6 ± 0.48	63.84 ± 0.28	99.93 ± 0.34	81.32 ± 0.50	59.56 ± 0.38	34.30 ± 0.67	10.49 ± 0.48^{b}	9.42 ± 0.47	14.50 ± 0.43	0.72 ± 0.03^{a}
GG	46	140.1 ± 0.58	64.22 ± 0.33	99.87 ± 0.41	80.79 ± 0.60	58.85 ± 0.45	34.02 ± 0.81	10.38 ± 0.58^{b}	9.46 ± 0.57	14.37 ± 0.53	0.72 ± 0.04^{a}
		NS	NS	NS	NS	NS	NS	$P < 0.01$	NS	NS	$P < 0.01$

N – number of experimental bulls. CL – carcass length. RL – rump length. RW – rump width. CW – chest width. ICD – inner chest depth. L^* (lightness), a^* (redness), b^* (yellowness), C^* (chroma), h^* (hue angle) – meat colour parameters. NS – not significant. [a,b,c] Different superscripts within a column indicate significant difference.

Table 5. Least-squares means, and standard errors for the significant associations of genotypic interactions with related traits in a Holstein population ($N = 400$).

CAST S20T × *LEP* A80V[a]			*CAPN1* V530I × *GHR* S555G[b]			*LEP* A80V × *GHR* S555G[b]			*CAPN1* V530I × *CAST* S20T[b]		
Genotype	*N*	DP (%)	Genotype	*N*	pH	Genotype	*N*	pH	Genotype	*N*	RW (cm)
CCCC	**19**	**54.33 ± 0.53**	AAAA	6	5.57 ± 0.06	CCAA	35	5.59 ± 0.03	AACC	5	94.74 ± 1.28
CCCT	29	54.02 ± 0.48	AAGA	3	5.54 ± 0.10	CCGA	14	5.55 ± 0.05	**AAGC**	**4**	**101.13 ± 1.03**
CCTT	77	53.13 ± 0.35	**AAGG**	**3**	**6.56 ± 0.17**	CCGG	10	5.83 ± 0.08	AAGG	3	100.04 ± 1.33
GCCC	31	53.07 ± 0.46	GAAA	61	5.57 ± 0.03	CTAA	55	5.52 ± 0.03	GACC	43	100.42 ± 0.39
GCCT	44	53.08 ± 0.40	GAGA	37	5.63 ± 0.03	CTGA	16	5.60 ± 0.04	GAGC	64	100.50 ± 0.36
GCTT	133	53.34 ± 0.35	GAGG	23	5.68 ± 0.04	**CTGG**	**10**	**6.09 ± 0.09**	GAGG	12	100.09 ± 0.66
GGCC	9	52.20 ± 0.72	GGAA	187	5.56 ± 0.02	TTAA	164	5.59 ± 0.03	GGCC	77	99.83 ± 0.33
GGCT	8	51.75 ± 0.75	GGGA	60	5.55 ± 0.03	TTGA	70	5.57 ± 0.04	GGGC	140	100.23 ± 0.31
GGTT	50	53.38 ± 0.42	GGGG	22	5.67 ± 0.04	TTGG	26	5.99 ± 0.06	GGGG	52	100.53 ± 0.40

N – number of experimental bulls. DP – dressing percentage. RW – rump width. [a] $P < 0.05$, [b] $P < 0.01$.

of the animals and the production procedures determine the slaughter weight and carcass traits (Sañudo et al., 2004) and inconsistent results about the associations between the same genetic markers with these traits can be evaluated as a common circumstance. Apart from these associations, carcass measurements (CL, RL, RW and ICD), BFT and LTL area were differentiated in the *CAPN1* G316A genotypes in the current study. Animals with the CC genotype had significantly lower values of the traits mentioned above. In the literature, associations of *CAPN1* G316A marker with carcass and growth traits have been shown in various cattle populations (Miquel et al., 2009; Pintos and Corva, 2011). To the best of our knowledge, this is the first study indicating that a portion of the differences in live and carcass weight and growth traits associated with this marker may be dependent on the body size (according to carcass measurement) of the individual. In the current study, animals with the CC genotype had 11.58 and 11.03 cm^2 lower mean value for LTL area, compared to GG and GC genotypes, respectively. These results indicated that selecting animals with the GG genotype induced higher values of LW, carcass weights and measurements and moreover higher BFT and LTL area as well. This knowledge may be useful for marker-assisted selection programmes. The trend of beef production in many countries has gradually changed from meat yield to meat quality. However, evaluating the ways to improve meat yield may be strategically important to achieve significant economic benefits in the countries with meat production deficit. Dairy-type animals, which yield a higher percent lean and less fat meat when compared with conventional beef breeds, could be exploited more commonly for beef production (Ntunde et al., 1977).

The CC genotype at the *CAPN1* G316A marker was absent or the frequency was rather low to estimate their association with phenotypic traits in several studies conducted on various cattle populations (Curi et al., 2010; Soria et al., 2010; Allais et al., 2011; Li et al., 2013). However, satisfying results were obtained for the frequencies of the CC genotype (0.06) and the C allele (0.28) in the current study. Bovine *CAPN1* has been mapped to the telomeric end of BTA29 (Smith et al., 2000; Page et al., 2002), including considerable overlap of QTLs regulating not only beef tenderness but also growth (weaning weight, carcass weight) and feed efficiency (Casas et al., 2003; Pintos and Corva, 2011). Hence, it is possible to obtain novel genetic associations among these traits by evaluating this genomic region.

The amino acid variations may cause a functional change in the μ-calpain protease. The μ-calpain isoform including V530I and G316A, or both, may be a functional protein variation in myofibrillar proteolysis and resulted in differences in meat quality (Page et al., 2002). The present results indicated that the *CAPN1* V530I marker was significantly associated with meat pH and L^* values. Choosing animals with the AA genotype at *CAPN1* V530I marker may have resulted in a selection of animals with lower L^* but higher pH values. Improper meat values of pH > 5.8 were observed for the AA genotype. It is worth noting that ultimate pH is one of the most important indices of meat quality and high quality-meat has ultimate pH at the range of 5.4–5.6 (Pipek et al., 2003). Moreover, the correlations between meat pH and all colour parameters proved to be significant. For example, the increase in meat pH may cause the deterioration of all colour parameters (Węglarz, 2010). Additionally, environmental conditions that, for example, cause additional stress on animals in the pre-slaughter period lead to high postmortem pH values and should be avoided (Pipek et al., 2003; Węglarz, 2010). Further experiments with larger populations should be conducted in order to evaluate the consequences of selection for the marker on optimum meat pH and quality, especially for the colour parameters.

The present results indicated that the *CAST* S20T polymorphism was associated with LW and ICD in Holstein bulls ($P < 0.05$). Animals with the GG genotype displayed a higher mean LW and ICD than those with the CC and heterozygous genotypes. Studies on the association of the *CAST* S20T polymorphism with LW and carcass measure-

ments in cattle are insufficient and larger populations may be needed to perform an adequate evaluation. Apart from the associations mentioned above, the b^* value indicating the degree of yellow appearance of meat from GC animals was higher than that estimated in the meat from those with other genotypes ($P < 0.05$). Consistent with our results, Juszczuk-Kubiak et al. (2004) reported that the meat from GC bulls had higher b^* values and was definitely darker. One possible connection between the *CAST* marker and meat colour may be through the calpain proteolytic system. The polymorphisms in genes related to calpain/calpastatin activity might affect meat colour traits, directly or indirectly (Li et al., 2013). Moreover, assessment of epistasis, genetic linkage and pleiotropy may be useful to consider different combinations of the polymorphisms associated with economically important quantitative traits.

The polymorphism A80V of the *LEP* gene has been reported as an effective marker on weight and average daily body weight gains (Kulig and Kmieć, 2009), marbling and carcass traits in feedlot cattle (Geary et al., 2003; Silva et al., 2014). However, there was no association between the *LEP* A80V polymorphism and any of the phenotypic traits evaluated in the current study. Studies on the association of the *LEP* A80V polymorphism with meat and carcass traits were conducted mostly in beef cattle populations. The reason for the lack of corresponding result in the present study may be the breed type.

Growth hormone influences growth and metabolism by interacting with GHR. The polymorphism S555G in exon 10 of the *GHR* gene has previously been shown to be associated with meat quality (Di Stasio et al., 2005; Reardon et al., 2010), growth and body size (Waters et al., 2011). Here, this SNP was associated with meat pH and meat colour parameters (a^* and h^*). Animals with GG genotype had higher pH values compared to the alternative genotypes. Conversely, Reardon et al. (2010) and Ribeca et al. (2010) found no association between this marker and meat pH. Environmental factors and pre-slaughter conditions of the abattoir may offer an explanation for variation in pH values reported in the different studies. The statistical analysis revealed that the effect of the GG genotype was significantly greater than the AA and heterozygote genotypes and that selection of animals with GG resulted in higher pH. In the study by Reardon et al. (2010), association of this marker at the *GHR* gene with L^* values of LTL and semimembranosus muscle was observed but a^* and b^* values were not differentiated between the *GHR* genotypes. Conversely, in this study, significant associations were found between *GHR* and a^* and h^* values but not L^* value. Meat derived from animals with AA genotype seemed to have higher a^* values (darker red colour) compared to those with other genotypes. One possible explanation about this association may be the effect of meat pH on colour parameters. High postmortem pH values influence meat colour negatively (Węglarz, 2010). In this study, AA genotype exhibited low pH and higher red values. Such genotypic information may have potential for incorporation into management systems for meat quality.

In the current study, we hypothesized that interactions between polymorphisms of the selected genes may have significant effects on meat yield and quality and, thereby, may provide novel perspectives to evaluate the availability of these polymorphisms. In this case, the interactions among *CAPN1*, *CAST*, *LEP* and *GHR* genotypes were investigated to acquire possible associations between genotypic combinations and phenotypic traits. Among these, the *LEP* A80V × *CAST* S20T was associated with DP ($P < 0.05$). Animals with the CCCC genotype exhibited the highest value of DP. Our results suggested that the individual genetic effects of these SNPs were not statistically significant for DP. Interestingly, the combined effects of these polymorphisms indicated a significant association in the interaction analysis. Evaluating non-allelic gene interactions and linkage in the corresponding genomic regions may be required before considering them in marker-assisted selection. The *CAPN1* V530I × *GHR* S555G and the *LEP* A80V × *GHR* S555G interactions were associated with variation in pH values ($P < 0.01$). Animals with the AAGG genotype of the *CAPN1* V530I × *GHR* S555G and animals with CTGG genotype of the *LEP* A80V × *GHR* S555G had very high pH values (6.56 and 6.09, respectively). High ultimate pH of meat can result in DFD (dark, firm, dry) meat occurrence. Moreover, high pH is improper for sorting, confectioning and vacuum packaging of meat (Pipek et al., 2003; Węglarz, 2010). Selecting animals with these genotypes may have an inadequate impact to commercial markets. In addition, AAGC animals of the *CAPN1* V530I × *CAST* S20T exhibited significantly higher means for RW compared to alternative genotypes ($P < 0.01$). However, investigation of a larger number of animals would be desirable, especially because of the genotypes with low frequency.

Information of SNPs in *CAPN1*, *CAST*, *LEP* and *GHR* genes may provide very important clues on how many and which polymorphisms can explain genetic variations in carcass characteristics and meat quality to produce constant meat products as well as to maintain commercial lines. In the current study, the *CAPN1*, *CAST* and *GHR* genotypes confirmed significant associations with important traits in adequate numbers of animals. Thus, G316A and V530I markers in the bovine *CAPN1* gene can be used as genetic markers in breeding programmes to improve meat quantity and quality traits, respectively. Similarly, *GHR* S555G and *CAST* S20T markers may be evaluated in conventional selection procedures, regarding improvements of meat colour and carcass traits.

Holstein cattle, which are bred mainly for dairy purposes, carry a potential for improvement of beef production due to their genetic variability for beef traits. Therefore, the dual capacity of the Holstein breed may be used in several countries to cover the shortage in milk and meat production (Calo

et al., 1973). Further genetic studies should be conducted for efficient selection procedures.

Consequently, information of polymorphisms at coding regions of the mentioned genes, genotypic interactions and significant genetic associations may be used to control meat production traits, concerning improvement of the genotypic structure.

5 Conclusions

This study focused on the associations of markers in the *CAPN1*, *CAST*, *LEP* and *GHR* genes with meat yield and quality traits. The present results confirm that the variation in these traits is influenced by the corresponding genotypes, except *LEP*. *CAPN1* G316A, a candidate marker for meat tenderness, also showed association with live weight and carcass characteristics. In addition, novel associations between genotypic interactions and phenotypic traits were observed. Further studies in larger populations consisting of various breeds with different genomic backgrounds should be conducted to evaluate valuable and useful marker associations.

Data availability. The original data are available upon request from the corresponding authors.

The Supplement related to this article is available online at https://doi.org/10.5194/aab-60-303-2017-supplement.

Author contributions. SA, FB and HS designed the study and wrote the paper. SA, HS and DD performed the experiments. SA, BS and DD collected the samples. FB and SA analysed the data and did the statistical analysis.

Competing interests. The authors declare that they have no conflict of interest.

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