Σχέση των γονιδίων βοοειδών IGF1, TG, DGAT1 και MYF5 με χρώμα του κρέατος, την τρυφερότητα και τις απώλειες του μαγειρέματος

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Relationship of the bovine *IGF1*, *TG*, *DGAT1* and *MYF5* genes to meat colour, tenderness and cooking loss

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ABSTRACT. Bovine insulin-like growth factor 1 (*IGF1*), thyroglobulin (*TG*), diacylglycerol-O-acyltransferase 1 (*DGAT1*) and myogenic factor 5 (*MYF5*) genes play an important role in the physiology of lipid and muscle metabolism and are therefore considered as candidate genes for meat production traits in farm animals. The objectives of this study were to investigate single nucleotide polymorphisms (SNPs) in *IGF1*, *TG*, *DGAT1* and *MYF5* genes and to evaluate whether these polymorphisms affected meat colour, tenderness and cooking loss in Holstein cattle. Initially, the SNPs were detected by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) method. Meat samples (N= 50) derived from *M. longissimus thoracis et lumborum* (LTL) were used in the current study. Significant differences in variations of meat colour parameters were observed at 24 hours post-mortem. *IGF1* was associated with colour parameters of a* and chroma values. In addition, effects of *TG* were statistically significant on L* and a* values, while, effects of *MYF5* were significant on a* value. There was no association of the tested SNPs with meat pH, tenderness and cooking loss. The results presented here may give the valuable information for improving meat colour in cattle.

*Keywords***:** Genetic markers, meat quality, single nucleotide polymorphisms, marker-assisted selection

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INTRODUCTION

The trend of beef production in many countries has gradually changed from meat yield to meat quality (Sañudo et al., 2004; Li et al., 2013). Carcass and meat quality traits, which are under the control of polygenic inheritance, are economically important traits and because meat quality is defined by the traits that consumer perceives as desirable (Warner et al., 2010). Among the characteristics considered, meat colour and tenderness influence the consumer's satisfaction about the quality of a piece of meat and determine the price accordingly (Grunert et al., 2004; Mullen et al., 2006; Li et al., 2013). Several factors have been identified when trying to evaluate meat colour and tenderness such as breed of animal, slaughter age, pre- and post slaughter conditions, aging process, meat pH, amount of connective tissue and packaging method (Priolo et al., 2001; Warner et al., 2010; Li et al., 2013; Frylinck et al., 2015). Apart from these environmental factors, many studies have investigated genetic effects on the mentioned traits and on the other hand, several genes associated with meat quality have been identified and single nucleotide polymorphisms (SNPs) of many candidate genes have been determined to be highly effective markers in beef production (Warner et al., 2010; Li et al., 2013).

Bovine insulin-like growth factor 1 (*IGF1*) gene is localized in chromosome 5 and consists of 6 exons (Bishop et al., 1991; Miller et al., 1992). *IGF1* has been shown to be a candidate gene for growth rate and meat production traits due to its role in regulation of cell proliferation and animal growth (Siadkowska et al., 2006). A thymine/cytosine transition (T/C), also recognizable as RFLP-*Sna*BI, at position 472 in the 5'-noncoding region of the *IGF1* gene (GenBank Acc. No: AF210383) has been reported to be candidate marker for growth performance and carcass traits (Li et al., 2004; Curi et al., 2005b; Siadkowska et al., 2006). Thyroglobulin (*TG*) gene is the molecular regulator for the thyroid hormones including T3 and T4 which are known to affect adipocyte differentiation and lipid metabolism (Shin and Chung, 2007; Pannier et al., 2010). *TG* gene has been mapped to the centromeric region of bovine chromosome 14 (Casas et al., 2005) and has been associated with lipid metabolism and meat

quality traits in various cattle breeds (Barendse, 1999; Barendse et al., 2004; Gan et al., 2008). A cytosine/thymine (C/T) polymorphism at position 422 of the *TG* gene (GenBank Acc No: X05380) has been associated with marbling score (Shin and Chung, 2007) and back fat thickness (Moore et al., 2003; Casas et al., 2005). The diacylglycerol-O-acyltransferase 1 (*DGAT1*) gene (Gen Bank Acc. No: AY065621), maps to chromosome 14, encodes the microsomal enzyme DGAT1 which is a catalyzer in the triglyceride synthesis pathway (Li et al., 2013). A lysine/alanine amino acid substitution in exon 8, at amino acid position 232 (K232A) of *DGAT1* gene has been demonstrated to be associated with milk components (Banos et al., 2008; Hradecká et al., 2008; Cerit et al., 2014) and higher levels of intramuscular fat (IMF) content in *semitendinosus* muscle (Thaller et al., 2003; Pannier et al., 2010; Tait et al., 2014). The genes of the muscle regulatory factors (*MRF*) gene family including myogenin (*MYOG*), myogenic factor 5 (*MYF5*), myogenic differentiation 1 (*MYOD1*) and myogenic factor 6 (*MYF6*) also called *MRF4* or herculin. All four genes are composed of 3 exons and share homology (Bhuiyan et al., 2009). Among them, *MYF5*, mapped to bovine chromosome 5, was evaluated as a muscle-specific factor and its expression was associated with myoblast lineage (Kisacova et al., 2009). In previous studies, polymorphisms in *MYF5* gene have been reported to be associated with growth traits (Li et al., 2004; Chung and Kim, 2005; Zhang et al., 2007; Bhuiyan et al., 2009).

The candidate genes were selected for this study with the objectives to estimate the effects of polymorphisms at the *IGF1*, *TG*, *DGAT1* and *MYF5* genes which were previously hypothesised to be genetic factors influencing adipocyte and myoblast differentiation, lipid metabolism, IMF deposition in bovine muscle regulation that has noteworthy effects on meat quality traits (Barendse, 1999; Thaller et al., 2003; Siadkowska et al., 2006). In the literature there is a limited information about the effects of the mentioned genes on meat colour and tenderness. Hence the objective of the current study was to evaluate the association of polymorphisms at four candidate genes with meat colour, tenderness and cooking loss in Holstein bulls.

MATERIALS AND METHODS

Animals and sampling

All animals selected for this study belonged to the Pedigree Project of the Turkish Ministry of Food, Agriculture and Livestock, and Cattle Breeders Association. Ethical approval was received from Uludag University Local Ethical Committee of Animal Experiments (Grant No: 2010-03/05). A total of 50 meat samples derived from purebred Holstein bulls that were grown on a private farm in the South Marmara region with the same feeding conditions and slaughtered at 15-17 months of age were used in the present study. The mean pre-slaughter weight of the animals was 469.04±7.65 kg. Previous to slaughter, stunning method was applied with a captive bolt stunner. After slaughter, all of the carcasses were electrically stimulated for a duration of 30 s (60V), suspended through the Achilles tendon. The carcasses were kept in a room at 4°C overnight. 5 cm-thick meat samples were collected from the *M. longissimus thoracis et lumborum* (LTL) at the 12th-13th intercostal area at 24 h after slaughter. The samples were vacuum-packed and exposed to ageing period for 6-7 days at 2°C. In addition, 4 mL blood samples were collected in K_3EDTA tubes (Vacutest Kima, SRL, Italy) from the vena jugularis of each of the bulls for genomic DNA extraction.

Meat quality evaluation

Meat colour parameters with the CIELAB colour space system including L* (lightness), a* (redness) and b* (yellowness) values were evaluated using Minolta CR 400 colorimeter (Minolta Camera Co., 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. Colour measurements were repeated 3 times on the meat samples from a fat-free area and the average of these measurements was evaluated as the final value. Chroma value (C) was calculated as $(a^{*2}+b^{*2})^{1/2}$ and hue angle (H) as arctan (b*/a*). Initial measurements of colour parameters were performed 24 h after slaughter. Additionally, ultimate pH at 24 h postmortem was measured using a digital pH meter (Testo 205, Lenzkirch, Germany). After 6-7 days ageing period, meat colour was measured immediately after cutting,

after 1 h storage and after 24 h storage on cut surface of 2.5 cm thick samples from fat-free area (Ekiz et al., 2009).

In order to measure cooking loss (%), meat samples were firstly weighed (initial sample weight), repacked in vacuum, cooked in a waterbath at 75°C for 1 h and the samples were reweighed (cooked sample weight). Mean initial weight of the samples was 300.204 ± 9.14 g. Cooking loss (%) was evaluated from the percentage of weight loss of the cooked sample compared to initial sample weight by using the formula of (initial sample weight - cooked sample weight) / initial sample weight x 100 (Ekiz et al., 2009). After the measurement of cooking loss, cooked samples were used to determine shear force value.

Six rectangular cross section (parallel to the muscle fibres) subsamples (3 cm long and 1x1 cm surface area) removed from cooked samples were sheared across the widest dimension using an Instron Universal Testing Machine (Model 3343, Norwood, MA, USA) which was set to 150 mm/min crosshead speed and 50 kg force applied to the meat. An average of the values for six sub-samples was accepted as the Warner-Bratzler shear force (WBSF) value for that sample (Ekiz et al., 2012).

DNA isolation and genotyping

DNA extraction was performed by a phenol-chloroform method as described by Green and Sambrook (2012). The concentration range (ng/µl) and purity (absorbance at 260–280 nm) of the DNA samples was measured with a spectrophotometer (NanoDrop 2000c, Thermo Scientific, Wilmington, DE, USA). In the current study, genotyping of the SNPs in the *IGF1*, *TG*, *DGAT1* and *MYF5* genes was carried out by PCR-RFLP. Primers used, PCR conditions and corresponding restriction enzymes are shown in Table 1. The PCR amplification was performed in a total volume of 50 µL containing 33.5 µL dH_2O, 5 µL 10 x Buffer, 5 µL $MgSO_4$, 1 µL dNTPs (2.5 mM), 2.5 U *Taq* DNA polymerase (Biomatik, A1003-500 U, 5 U/µL), 1 µL (0.025 µM) of each primer and 3 µL of the DNA sample at a concentration of 100 ng/µL. PCR amplicons of the *IGF1*, *TG*, *DGAT1* and *MYF5* genes were subjected to restriction enzyme digestion with suitable restriction enzymes according to the

Table 1. Primers used, PCR conditions and corresponding restriction enzymes in the present study

Gene	PCR amplicon (bp)	Forward primer	Reverse primer	PCR conditions	Restriction enzyme	Reference
IGF1	249	5'ATTACAAAGCTGCCTGCCCC3'	5'ACCTTACCCGTATGAAAGGAATATACGT3'	94°C 5′ (94 °C 1′, 64°C 1′, 72°C 1′) 31 cycles, 72°C 5′	*Sna*BI	Siadkowska et al. (2006)
TG	545	5'GGGGATGACTACGAGTATGACTG3'	5'GTGAAAATCTTGTGGAGGCTGTA3'	94°C 5′ (94 °C 1′, 55°C 1′, 72°C 1′) 35 cycles, 72°C 7′	*Mfl*I	Shin and Chung (2007)
DGAT1	411	5'GCACCATCCTCTTCCTCAAG3'	5'GGAAGCGCTTTCGGATG3'	*94°C 4′, 10 cycles (94°C 60 s, 66°C 60s; −1°C per cycle, 72°C 60s) 25 cycles (94°C 60s, 56 °C 120 s, 72°C 60s), 72°C 15′	*Cfr*I	Lacorte et al. (2006)
MYF5	445	5'ACAGCGTCTACTGTCCTGATG3'	5'CGTGGTATATACTAAGGACAC3'	94°C 4′ (94 °C 30s, 58°C 60s, 72°C 60s) 38 cycles, 72°C 4′	*Taq*I	Kisacova et al. (2009)

IGF1: insulin-like growth factor 1, *TG*: thyroglobulin, *DGAT1*: diacylglycerol-O-acyltransferase 1, *MYF5*– myogenic factor 5 * Touch-down PCR protocol was performed.

suppliers instructions. Briefly, enzyme-buffer mix was prepared by mixing 2 μL of restriction enzyme with 8 μL of the respective buffer. Reaction mix was prepared by mixing 10 μL PCR product with 2 μL of enzyme buffer mix. Volume was made up to 20 μL with autoclaved MilliQ water. Afterwards, these reaction mixtures were incubated at 37°C for 16 h. The samples were put into the 3% agarose gel (Sigma Aldrich, Steinheim, Germany) with 1 x TBE buffer for electrophoretic separation. Ethidium bromide (1 μg.mL^{-1}) was used as an intercalated reagent. Electrophoresis ran at voltage max. 90 V per 1 h. Visualization of DNA fragments was made in using of gel imaging system (DNR-Minilumi, DNR Bio-Imaging Systems, Israel). As a reference to estimate the size of fragments we applied DNA ladder (100–1000 bp, Biomatik).

Statistical analysis

The genotype and allele frequencies of each SNP were calculated by the standard procedure (Falconer and Mackay, 1996). The Hardy–Weinberg equilibrium (HWE) was tested for all alleles by using the 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 by using the following formulas as described by Nei and Roychoudhury (1974) and Botstein et al. (1980):

$$He = 1 - \sum_{i=1}^{n} P_i^2$$

$$Ne = 1/\sum_{i=1}^{n} P_i^2$$

$$PIC = 1 - (\sum_{i=1}^{n} P_i^2) - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2P_i^2 P_j^2$$

Where:

P_i was the frequency of the ith allele,

n was the number of alleles.

Statistical analyses were carried out using SPSS v23.0 (IBM, Armonk, NY, USA). Levene's test was used to test for homogeneity of the variances. Meat colour parameters were considered as repeated measures and analyzed by using the Repeated Measures of General Linear Model (RM-GLM) procedures. Orthogonal polynomial contrasts were used to estimate the linear, quadratic and cubic effects of meat colour variation and time as well as their interactions. The mix models in the present study, as shown below, were selected by evaluating the adjusted R^2 to compare the explanatory power of models with different numbers of predictors.

$$Y_{ijklmno} = \mu + IGHF1_i + TG_j + DGAT1_k + MYF5_l + LW_m + S_n + \sum_{o=1}^{4} b_o X_{o_{ijklmno}} + e_{ijklmno}$$

Where:

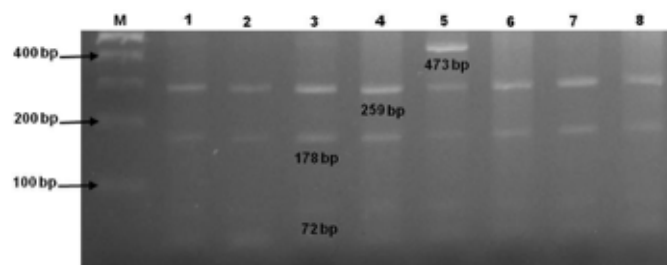$Y_{ijklmno}$ = the studied traits; μ = the overall mean;

$IGF1_i$ = the fixed effect of the *IGF1* genotype; TG_j = the fixed effect of the *TG* genotype; $DGAT1_k$ = the fixed effect of the *DGAT1* genotype; $MYF5_l$ = the fixed effect of the *MYF5* genotype; LW_m = the fixed effect of pre-slaughter live weight (m = <430, 431-490, 491<); S_n = the fixed effect of season at the slaughter (n = winter, spring, summer); b = regression coefficient; X_o = meat colour parameters measured at different times (o = 1: meat colour parameters at 24h post-mortem, 2: 0h after ageing, 3: 1h after ageing, 4: 24h after ageing). In addition, the model for the independent variables (meat pH at 24h post-mortem, shear force values and cooking loss) is as follows:

$$Y_{ijklmno} = \mu + IGHF1_i + TG_j + DGAT1_k + MYF5_l + LW_m + S_n + e_{ijklmno}$$
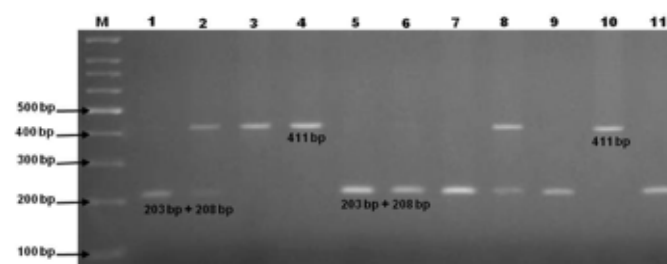
When significant associations were identified, the mean values for each effect were contrasted using Tukey's test.
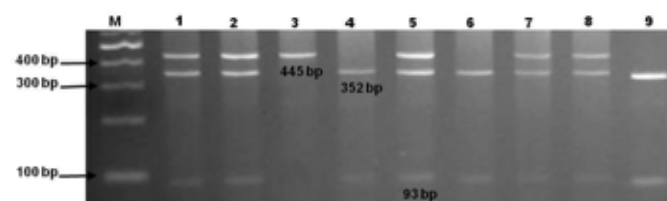
RESULTS

We have amplified the 249 bp fragment in the 5'-non-coding region of the *IGF1* gene. Digestion of the PCR product with the *Sna*BI nuclease resulted in two bands (223 bp and 26 bp) for homozygote TT and three bands (249 bp, 223 bp and 26 bp) for the heterozygote genotype. The DNA amplified from CC animals remained undigested with the corresponding restriction enzyme (Fig 1). The amplified fragment of the C422T polymorphism (545 bp) in *TG* gene showed three genotypes including CC, CT and TT by digestion with the restriction enzyme *Mfl*I and the C allele was cleaved into three bands of 72 bp, 178 bp and 259 bp, while T allele showed two bands of 72 bp and 473 bp (Fig 2). However, TT genotype was not

Figure 1. The electrophoresis pattern of C472T polymorphism within the bovine *IGF1* gene (M: Marker; Lanes 2 and 7: CC; Lanes 1,3,4,6,8 and 9: CT; Lane 5: TT).

Figure 2. The electrophoresis pattern of C422T polymorphism within the bovine *TG* gene (M: Marker; Lane 5: CT; Lanes 1-4 and lanes 6-8: CC; Note: TT genotype was not present).
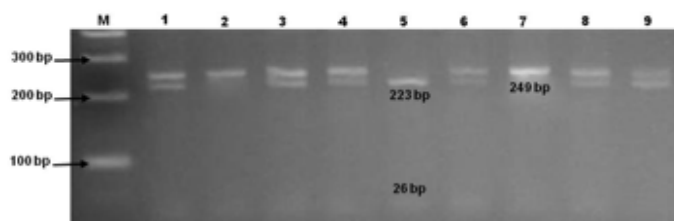

Figure 3 The electrophoresis pattern of K232A polymorphism within the bovine *DGAT1* gene (M: Marker; Lanes1, 5-7, 9,11: AA; Lanes 2 and 8: KA; Lanes 3, 4 and 10: KK).

Figure 4 The electrophoresis pattern of g.1911A>G polymorphism within the bovine MYF5 gene (M: Marker; Lanes 4,6 and 9: *Genotype* '22'; Lanes 1,2,5,7 and 8: Genotype '12'; Lane 3: Genotype '11').

present in the current study. The cleavage of a 411 bp PCR product by *Cfr*I yielded two fragments of 203 bp and 208 bp and was diagnostic for the AA genotype in the *DGAT1* assay. These two bands (203 bp and 208 bp) were observed as a single band (203 bp + 208 bp) in gel electrophoresis pattern. Heterozygote genotype was cleaved into three bands of 411 bp, 208 bp and 203 bp. Besides the DNA amplified from KK animals remained undigested with the *Cfr*I nuclease (Fig 3). Typing *MYF5* allelic variation by PCR-RFLP

Table 2. Allele and genotype frequencies of polymorphisms in *IGF1*, *TG*, *DGAT1* and *MYF5* genes, population genetic indices (He, Ne, PIC) and compatibility with the Hardy-Weinberg equilibrium.

SNP	*IGF1*			*TG*			*DGAT1*			*MYF5*		
Genotypes	CC	CT	TT	CC	CT	TT	AA	KA	KK	11	12	22
N	4	40	6	45	5	0	7	36	7	3	26	21
%	8	80	12	90	10	0	14	72	14	6	52	42
MAF		0.48			0.05			0.50			0.32	
He		0.4992			0.0950			0.5000			0.4352	
Ne		1.9968			1.1049			2.0000			1.7705	
PIC		0.3745			0.0904			0.3750			0.3405	
χ^2(HWE)*		18.15*			26.36*			9.68*			1.89	

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

bp when digested with *Taq*I restriction enzyme, while for allele 1, the 445 bp band was diagnostic. The presence of both alleles (heterozygote genotype: 12) was characterized by fragment sizes 445 bp, 352 bp and 93 bp (Fig 4).

The genotypic and allelic frequencies, population genetic indices including He, Ne and PIC and compatibility with the Hardy-Weinberg equilibrium (HWE) are shown in Table 2. Two alleles and three genotypes in each SNP were found in the current study, except for *TG* because TT genotype was not present. Results indicated that the population were determined not to be compatible for *IGF1*, *TG* and *DGAT1* genotypes in the Hardy-Weinberg equilibrium ($P<0.001$). The minor allele frequencies ranged from 0.05 to 0.50 were observed and all markers in the present study were polymorphic except for *TG*, according to the classification reported by Menezes et al. (2006). In addition to this, He values ranged from 0.0950 to 0.5000, PIC values ranged from 0.0904 to 0.3750 and Ne values ranged from 1.1049 to 2.0000 were observed in the chi-square statistics.

In Table 3, significant probability values obtained in the present study are given for linear, quadratic and cubic effects. Results indicated that, time effect on meat colour parameters was statistically significant except for linear effect on L* value, quadratic and cubic effects on a* value and cubic effect on chroma value. Time x *IGF1* interaction revealed linear effect on L*, a* and chroma value ($P<0.05$).

Table 3. Significant probability values obtained in the ANOVA

Effects	Meat colour parameters				
	L*	a*	b*	C	H
Time					
Linear	⋯	0.000	0.028	0.000	0.000
Quadratic	0.005	⋯	0.000	0.000	0.000
Cubic	0.020	⋯	0.000	⋯	0.000
Time x *IGF1*					
Linear	0.038	0.003	⋯	0.044	⋯
Quadratic	⋯	0.000	⋯	0.002	⋯
Cubic	⋯	⋯	⋯	⋯	⋯
Time x *TG*					
Linear	0.050	0.023	⋯	⋯	⋯
Quadratic	0.032	0.014	⋯	0.050	⋯
Cubic	⋯	⋯	⋯	⋯	⋯
Time x *DGAT1*					
Linear	⋯	⋯	⋯	⋯	⋯
Quadratic	⋯	⋯	⋯	⋯	⋯
Cubic	⋯	⋯	⋯	⋯	⋯
Time x *MYF5*					
Linear	⋯	0.027	⋯	⋯	⋯
Quadratic	⋯	⋯	⋯	⋯	⋯
Cubic	⋯	⋯	⋯	⋯	⋯
Time x Season					
Linear	⋯	⋯	⋯	⋯	⋯
Quadratic	⋯	⋯	⋯	⋯	0.049
Cubic	⋯	⋯	⋯	⋯	⋯
Time x Live weight					
Linear	⋯	⋯	⋯	⋯	⋯
Quadratic	⋯	⋯	⋯	⋯	⋯
Cubic	⋯	⋯	⋯	⋯	⋯

L*: lightness, a*: redness, b*: yellowness, C:chroma, H: hue angle.

Table 4. Least squares means (±standard errors) for *IGF1, TG*, *DGAT1* and *MYF5* genotype effects on meat colour parameters.

Parameters	IGF1 CC (N=4)	IGF1 CT (N=40)	IGF1 TT (N=6)	TG CC (N=45)	TG CT (N=5)	DGAT1 AA (N=7)	DGAT1 KA (N=36)	DGAT1 KK (N=7)	MYF5 11 (N=3)	MYF5 12 (N=26)	MYF5 22 (N=21)
(L*)S	33.80 2.10	31.53 1.37	34.21 1.98	35.93 1.14[a]	30.43 1.97[b]	32.50 1.85	33.66 1.42	33.38 1.97	34.18 2.50	33.83 1.17	31.53 1.28
(L*)0h	25.94 2.07	30.83 1.35	28.91 1.94	28.62 1.13	28.50 1.93	28.21 1.82	28.53 1.40	28.93 1.93	27.07 2.46	29.54 1.15	29.07 1.26
(L*)1h	26.47 2.38	31.97 1.55	29.88 2.24	29.43 1.30	29.45 2.23	29.03 2.09	29.24 1.61	30.06 2.22	28.16 2.83	30.10 1.33	30.06 1.45
(L*)24h	28.39 2.27	32.88 1.48	30.31 2.14	30.85 1.24	30.20 2.12	30.03 1.99	30.80 1.54	30.75 2.12	28.99 2.70	31.60 1.27	30.98 1.38
(a*)S	15.90 1.15[a]	11.074 0.75[b]	11.47 1.08[b]	11.60 0.63[b]	14.03 1.08[a]	13.35 1.01	12.91 0.78	12.19 1.08	12.25 1.37[ab]	12.22 0.64[b]	13.96 0.70[a]
(a*)0h	14.59 0.85	15.42 0.55	15.47 0.81	15.20 0.47	15.12 0.80	14.74 0.75	15.56 0.58	15.19 0.80	15.50 1.02	15.12 0.48	14.86 0.52
(a*)1h	17.44 1.26	18.18 0.82	18.60 1.19	18.41 0.69	17.73 1.18	17.98 1.11	18.49 0.85	17.75 1.18	18.63 1.50	17.97 0.70	17.61 0.76
(a*)24h	22.33 1.55	20.55 1.01	21.28 1.46	21.53 0.84	21.24 1.45	21.67 1.36	21.33 1.05	21.16 1.45	23.24 1.85	20.87 0.87	20.05 0.95
(b*)S	8.90 2.06	9.22 1.35	9.83 1.94	9.07 1.12	9.57 1.93	8.73 1.82	10.36 1.40	8.87 1.93	10.23 2.46	9.13 1.15	8.60 1.26
(b*)0h	-3.82 0.76	-2.24 0.49	-2.88 0.72	-2.98 0.41	-2.98 0.71	-3.05 0.67	-2.88 0.52	-3.00 0.71	-3.53 0.91	-2.56 0.43	-2.85 0.46
(b*)1h	-0.76 1.67	2.41 1.09	1.83 1.57	1.32 0.91	1.00 1.56	1.12 1.47	1.33 1.13	1.03 1.56	0.51 1.99	1.59 0.93	1.37 1.02
(b*)24h	2.91 1.42	4.53 0.93	3.51 1.34	3.87 0.78	3.43 1.33	3.22 1.25	4.01 0.97	3.72 1.33	3.18 1.70	4.19 0.79	3.58 0.87
(C)S	18.75 1.37[a]	14.72 0.89[b]	15.28 1.29[ab]	15.04 0.75	17.46 1.28	16.43 1.20	16.97 0.93	15.36 1.28	16.16 1.63	15.69 0.77	16.88 0.84
(C)0h	15.09 0.79	15.66 0.51	15.81 0.74	15.55 0.43	15.49 0.74	15.16 0.69	15.85 0.53	15.55 0.74	15.91 0.94	15.43 0.44	15.22 0.48
(C)1h	17.50 1.39	18.51 0.91	18.83 1.31	18.64 0.76	17.91 1.31	18.27 1.23	18.65 0.95	17.92 1.31	18.75 1.66	18.24 0.78	17.85 0.85
(C)24h	22.54 1.75	21.15 1.14	21.65 1.64	21.96 0.95	21.60 1.64	21.99 1.53	21.76 1.18	21.60 1.63	23.49 2.08	21.38 0.98	20.48 1.07
(H)S	0.52 0.14	0.69 0.08	0.71 0.13	0.66 0.07	0.62 0.13	0.59 0.12	0.68 0.09	0.65 0.13	0.71 0.16	0.65 0.07	0.56 0.08
(H)0h	-0.26 0.05	-0.15 0.04	-0.19 0.05	-0.19 0.03	-0.21 0.05	-0.21 0.05	-0.19 0.04	-0.19 0.05	-0.23 0.07	-0.17 0.03	-0.19 0.03
(H)1h	-0.05 0.09	0.11 0.06	0.08 0.08	0.06 0.05	0.04 0.08	0.04 0.07	0.06 0.06	0.05 0.08	0.01 0.11	0.07 0.05	0.06 0.05
(H)24h	0.11 0.06	0.20 0.04	0.15 0.056	0.17 0.03	0.14 0.05	0.12 0.05	0.17 0.04	0.16 0.05	0.11 0.07	0.19 0.03	0.16 0.04

L*: lightness, a*: redness, b*: yellowness, C: chroma, H: hue angle

S: represents colour parameters measured at 24h post-slaughter, 0h, 1h, 24h: represents colour parameters measured at 0h, 1h and 24h after ageing period

[a,b] Different superscripts within a raw indicate significant difference

significantly affected the linear and quadratic terms of fitted meat colour development curves. Time x *MYF5* interaction revealed a significant effect on the linear term of the a* value (*P*<0.05). Besides, phenotypic interactions including time x season and time x live weight were evaluated in the current study. However, only quadratic term of the time x season interaction was observed (*P*<0.05).

Least squares means (±standard errors) for *IGF1*, *TG*, *DGAT1* and *MYF5* genotype effects on meat colour parameters are shown in Table 4. The results indicated that *IGF1* was associated with colour parameters of a* and chroma value at 24 h post-slaughter (*P*<0.05). Meat derived from CC animals had higher values of mentioned colour parameters compared to alternative genotypes. In the present study the effects of *TG* was statistically significant on

L* and a* values (*P*<0.05). In this respect, on the one hand meat from CC animals seemed to be brighter in colour; on the other hand meat from heterozygous animals was redder. In addition, a significant association was observed between the *MYF5* marker and a* value at 24h post-mortem. The '22' genotype seemed to be associated with redder meat compared to alternative variants of the polymorphism (*P*<0.05). There was no association between any of the tested SNPs with meat colour parameters after ageing period, nor was there any association with variation in pH, tenderness and cooking loss, as shown in Table 5 (*P*>0.05).

DISCUSSION

In this work, the result of Hardy-Weinberg disequilibrium (*P*<0.001) in *IGF1*, *TG* and *DGAT1* genotypes

Table 5. Least squares means (±standard errors) for *IGF1*, *TG*, *DGAT1* and *MYF5* genotype effects on pH at 24h post-mortem, shear force and cooking loss.colour parameters.

Parameters	Genotype										
	IGF1			TG		DGAT1			MYF5		
	CC (N=4)	CT (N=40)	TT (N=6)	CC (N=45)	CT (N=5)	AA (N=7)	KA (N=36)	KK (N=7)	11 (N=3)	12 (N=26)	22 (N=21)
pH (24h)	5.94 0.14	5.88 0.09	5.76 0.14	5.74 0.07	5.98 0.14	5.82 0.13	5.83 0.11	5.93 0.14	5.91 0.18	5.90 0.08	5.78 0.09
WBSF (kgf/cm2)	3.75 1.31	6.16 0.85	4.22 1.23	4.92 0.71	4.5 01.22	4.15 1.15	5.26 0.88	4.71 1.22	6.71 1.56	3.72 0.73	3.69 0.79
CL (%)	21.01 3.36	24.92 2.20	22.0 03.16	23.00 1.83	22.29 3.15	21.84 2.96	25.60 2.28	20.49 3.15	24.39 4.01	21.78 1.88	21.77 2.05

WBSF: Warner-Braztler shear force, CL: cooking loss

may have been due to limited sample sizes from the population and artificial selection of parents for the milk production traits that should be considered for Holstein breed. According to the classification of PIC values (PIC<0.25= low informative polymorphism; 0.25<PIC <0.50= intermediate/mildly informative polymorphism; PIC>0.50= high informative polymorphism), population had intermediate levels of genetic diversity (Botstein et al., 1980). However, *TG* may not be included to the mentioned classification because of the T allele with low frequency (0.05) resulting in low genetic variabilities of He, Ne and PIC.

Meat colour is an extremely influential trait for commercial markets. Although colour is only slightly correlated with the eating characteristics of meat, it is very important for the consumer's choice (Priolo et al., 2001; Modika et al., 2015). Recently, many studies have investigated genetic effects on meat colour, however, testing of the SNPs in candidate genes may provide novel aspects to genetic background of meat quality. Based hereupon, we aimed to assess novel associations between *IGF1*, *TG*, *DGAT1* and *MYF5* genes and meat colour at pre- and post ageing process. Among them, the polymorphism in *IGF1* was significantly associated with colour parameters of a* and chroma values at 24 h post-mortem (*P*<0.05). The CC genotype seemed to be favourable in the present study. However, there was no association between the *IGF1* and any of the colour parameters after 6-7 days of ageing period. Curi et al. (2005a) observed significant associations of *IGF1* genotypes with body weight and subcutaneous back fat, and the

effects of these genotypes on *M. longissimus thoracis et lumborum* area tended to be significant in different genetic groups of beef cattle.

Moreover, Li et al. (2004) reported that, CC genotype of *IGF1* was associated with higher live body weight at weaning in commercial lines of *Bos taurus*. As shown by Siadkowska et al. (2006) the CC genotype was found favourable also for feed consumption and conversion. In the literature, there is insufficient information about the association between the *IGF1* C472T marker and variation in meat colour to draw conclusions. Previous studies indicated that, effective quantitive trait loci (QTL) for meat production traits (Casas et al., 2000; MacNeil and Grosz, 2002) were assigned to bovine chromosome 5 (BTA5), where the *IGF1* gene is located. Our results suggest that focusing on this genomic region may be useful in improving not only growth traits but also meat colour.

Thyroid hormones play an important role in regulating adipocyte growth and differentiation, fat depots and homeostasis of the entire lipid metabolism. *TG* is the molecular precursor for thyroid hormones (Casas et al., 2005). Hence, *TG* is considered as a functional candidate gene as well as a positional candidate and is widely used in marker assisted selection programs to improve meat quality (Shin and Chung, 2007). In the literature, *TG* C422T polymorphism was considered as an effective marker for marbling scores (Barendse et al., 2001; Grisart et al., 2002; Burrell et al., 2004; Shin and Chung, 2007), *longissimus* muscle area (Casas et al., 2005), intramuscular fat content in *M. longissimus thoracis et lumborum* (Thaller et al., 2003) and back fat thick-

ness (Casas et al., 2005) in various cattle populations. We report herein the results of a study in which we evaluated how appropriately *TG* C422T polymorphism influenced meat colour parameters (linear and quadratic terms). Meat samples derived from animals with genotype CC had significantly higher L* values, but lower a* values at 24h post-mortem compared to those with heterozygous genotype ($P<0.05$). Visual appraisal of meat colour may also be affected by marbling and a large amount of intramuscular fat can increase lightness (Fiems et al., 2000). One possible explanation about the existence of the association between *TG* and meat colour parameters observed in the current study may be through intramuscular lipid deposition. The relatively small number of meat samples involved in the present study does not allow to draw final conclusions about the effects of *TG* C422T polymorphism on meat colour. However, it would be particularly interesting to evaluate the novel associations at the corresponding locus in future studies.

MYF5, which is included in the *MYOD* gene family, was considered as a candidate gene for meat production traits due to its potential roles in proliferation through muscle fiber development (Bhuiyan et al., 2009). Results of the gene-specific SNP marker association analysis for the *MYF5* reported by Li et al. (2004) and Bhuiyan et al. (2009) indicated that, g.1911A>G SNP genotypes of *MYF5* gene had significant effect on average daily gain in Canadian beef cattle and Korean cattle (Hanwoo) respectively. Moreover, similar results were observed in the studies performed by Chung and Kim (2005) and Zhang et al. (2007). To the best of our knowledge, there is no information about the association of *MYF5* g.1911A>G polymorphism with meat colour parameters. Our results revealed that the genotype '22' was significantly associated with redder meat (higher values of a*) and seemed to be favourable ($P<0.05$). Nevertheless, no significant difference was obtained for the quadratic and cubic nonlinear terms. *MYF5* was mapped to BTA5 including considerable overlap of QTLs regulating not only growth traits but also meat quality (Casas et al., 2000). Moreover, muscle fibre characteristics may influence meat colour characteristics, although a few reports exist in cattle (Vestergaard et al., 2000). In this respect, the

results of the current study may provide an alternative aspect for future studies.

DGAT1 gene, mapped to BTA14, is functional and positional candidate gene for traits related to fat synthesis in both dairy and beef cattle (Curi et al., 2011). However, there was no association between the *DGAT1* K232A polymorphism and any of the phenotypic traits evaluated in the current study. In addition, there was no association of the tested SNPs with meat pH at 24h post-mortem, WBSF values and cooking loss ($P>0.05$). Meat quality traits such as tenderness and cooking loss are regulated by many genes and affected by interactions among them, and thus, a candidate gene associated with a trait in one population may have a different effect, or show no effect at all, in another population due to negative effects of other genes and epistatic interactions of the candidate gene with other genes in the population (Ge et al., 2003). Hence, inconsistent results about the associations between the same genetic markers and the mentioned traits can be evaluated as a common circumstance. On the other hand, indirect selection of the mentioned loci from the selection for milk production should be considered for Holstein breed.

CONCLUDING REMARKS

This study focused on the associations of markers in the *IGF1*, *TG*, *DGAT1* and *MYF5* genes with meat colour, tenderness and cooking loss. Novel associations between the *IGF1*, *TG* and *MYF5* genotypes and meat colour parameters traits were observed. The present results could therefore be indicative for future studies on meat quality, especially colour evaluation, in livestock.

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CONFLICT OF INTEREST STATEMENT

None of the authors of this article has any conflict of interest.

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