International Food Research Journal 30(1): 130 - 141 (February 2023)
Journal homepage: *http://www.ifrj.upm.edu.my*

Effects of leptin and thyroglobulin gene polymorphisms on beef colour in Holstein bulls for slaughter in Turkey

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Article history

Received:
29 December 2021
Received in revised form:
14 April 2022
Accepted:
7 June 2022

Keywords

Holstein,
hot carcass weight,
beef quality,
SNP,
meat colour

Abstract

In the present work, live weight (LW), hot carcass weight (HCW), and beef colour values of Turkish Holstein bull (THBs) samples, and their relationship with single nucleotide polymorphisms (SNPs) variants were determined. E2JW and E2FB variants of leptin (LEP), and C422T variant of thyroglobulin (TG) genes were determined in 100 heads of THBs by polymerase chain reaction fragment length polymorphism (PCR-RFLP). Genotyping was carried out by capillary electrophoresis. The colour of raw and cooked beefs was spectrophotometrically measured before and after cooking. The cooked beefs were significantly brighter in the LEP E2JW AA and AT variants than in the TT genotype ($p < 0.05$). Based on b* of raw beefs, the yellowish colour density in the LEP E2JW AA genotype variant was significantly higher than in AT and TT ($p < 0.05$). The most significant correlation was determined between b* and L* (0.695), and b* and a* (0.694) of raw beefs, while the correlation coefficient between LW and HCW was found to be 0.604 ($p < 0.01$). The LEP E2JW AA marker genotype for cattle with brighter and more intense beef, and the LEP E2JW TT variant genotype to increase beef yield should be selected as a study by using MAS method at an early age. Also, AT / CT / CC marker genotypes of THBs should be selected in LEP E2JW / E2FB / TG C422T marker loci, respectively to generate more income from the increase in LW and HCW.

DOI
https://doi.org/10.47836/ifrj.30.1.10

Introduction

Colour is one of the most important characteristics of fresh meat that affect consumers' preference (Font-i-Furnols and Guerrero, 2014; Hughes *et al.*, 2014). When a consumer decides to buy beef, one first chooses the product that pleases one's eye. Therefore, beef colour is an important quality approach in determining whether consumers will buy the product (Suman *et al.*, 2014; Kiran *et al*., 2018).

Colour of meat is inherited, and important in determining meat quality. Meat contains myoglobin (muscle) and haemoglobin (blood) colour pigments. The amount of pigment has special importance in the formation of meat colour (Girolami *et al.*, 2013). Beef colour also influences the brightness of the red muscles, which is related to the pigment content of myoglobin (Hughes *et al.*, 2014). The task of myoglobin in the muscle is to store oxygen (O_2). O_2 binds to myoglobin, and turns it into oxymyoglobin, and the colour becomes cherry red. This is perceived as fresh beef. High-density beef in terms of myoglobin is dark red, while low-density beef is light red (Lawrie, 2006; Girolami *et al.*, 2013; Suman *et al.*, 2014). The amount of myoglobin in beefs varies based on gender, species, age, nutrition, slaughter factors, physical activity, and body regions (Suman and Joseph, 2013).

Meat surface colours vary due to the reactions of myoglobin (Suman *et al.*, 2014). By increasing the activities of oxidative enzymes in the muscles, metmyoglobin is formed in the muscles by using the necessary O_2 for oxymyoglobin. For this reason, to reduce the activities of these enzymes, the carcasses must be kept in cold storage after slaughter, and shredded in that same environment. As a result of microbial enzyme activity, the lactobacilli shred the

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haemoglobin, and turn the meat colour into a green colour. The green colour that occurs due to microbial reproduction can turn brown or even yellow due to oxidation which causes the meat to rot. Metmyoglobin, which is a brown pigment, is an undesirable colour pigment that causes the discoloration of beef. Depending on the denaturation, the colour of the meats may change from dull red to brown during cooking (Lawrie, 2006; Girolami *et al.*, 2013).

Many methods are used to measure meat colour; the most accurate and precise being spectrophotometry and colorimetry (Trinderup *et al.*, 2015; Broadbent, 2017). To analyse colour in meat, a colour identification system namely L* (brightness), a* (red colour index), and b* (yellow colour index) colour coordinates was used (Murray, 1995). In evaluating beef colour, the musculus longissimus dorsi (MLD) is determined as a reference in many countries, and the colour standard of the beef is evaluated on this muscle (Taşçı, 2017). Various studies on beef colour in cattle exist in the literature (De Oliveira *et al.*, 2013; Papaleo Mazzucco *et al.*, 2016; Ardıçlı *et al.*, 2018). There is a relationship between the physical variables of beef colour and other beef quality characteristics. Since the colour measurement is an easy and fast method, correlations (*r*) are used to estimate the deterioration in beef quality. Therefore, it is important to know the *r*-relationships between meat colour measurements and other beef quality characteristics. L*, a*, and b* and percentages of pigment on the meat surface can also be used to determine the shelf life of beef stored in different atmospheres (Insausti *et al.*, 2008).

The growth of cattle is represented by the increase in LW from birth to standard life stages (Forni *et al.*, 2007). HCW is determined by weighing the beef with bones in sets consisting of legs and rumps before being put into cold storage. LW is the live weight of the animals determined by earring numbers before slaughter. LW is affected by genetic and environmental sources of variation (Krupa *et al.*, 2005). Thanks to deoxyribose nucleic acid (DNA) technology in bovine, many marker genes associated with LW traits have been identified (De Carvalho *et al.*, 2012). Among these genes, LEP and TG genes play an essential role in beef quality and yield (Casas *et al.*, 2005; De Oliveira *et al.*, 2013; Kök *et al.*, 2015). LEP and TG genes are considered as potential candidate genes in quantitative trait locus (QTL) based selection programs for beef quality improvement of cattle breeds, and an attempt has been made to determine their relationship with beef colour in THBs. Males of Holstein breeds are also used in beef production for butchery due to their rapid development. We aim to determine the bulls in the marker genotype, which positively affects the beef quality and yield of the THB breed, which is grown in beef production, by utilising QTL without being affected by environmental conditions. Thus, by using LEP and TG genes related with beef production, a reference population will be established for THBs under intensive growing conditions in Turkey. In addition, using marker genotypes (LEP E2JW / E2FB / TG C422T) and phenotypic relationships determined in these THB samples will contribute to better quality beef production with marker-assisted selection (MAS) in the entire THB population in Turkey. Also, classifying calves whose marker genotype is determined at a young age while the animals producing quality meat before slaughter are alive, and evaluating the ones for breeding and slaughter with MAS, will contribute to their sale. In addition, it will make the consumption of quality beef sustainable by consumers.

Materials and methods

Animals and beef samples

Our research was carried out on the beef quality, beef production potential, and yield of 17-month-old Holstein bulls, which are the most widely used in beef production and consumption in Edirne, Turkey. The six-month-old male calves of Turkish Holstein cattle were collected from different cattle farms in Kırklareli and Edirne. They were fed intensively with granulated grain, pulp, and roughage such as wheat straw, dry clover, and corn silage for an average of one year, under similar conditions in two semi-open farms in Edirne. After fattening, the bulls were slaughtered in the Edirne Commodity Exchange Slaughterhouse in December 2017 by the vertical cut method. To generate genotypic diversity, 100 heads of THB brought for slaughter from these two farms constituted the research samples. The altitude of the two farms in Edirne was 134 ft (41 m). LW measurements of cattle were made before slaughter, and HCW measurements were made after slaughter. The ribeye samples were removed (about 1 kg, including the MLD muscle between the 12^{th} and 13^{th} ribs) from the carcasses, and left to rest for 24 h at +4°C after slaughter, with the help of the butcher.

The molecular analysis and the colour analyses of the THB beef were made from the MLD muscle samples (Pogorzelska *et al*., 2013).

Colour analysis

For the colour analysis of raw and cooked beefs, three pieces of cubic MLD beef was cut to the thickness of 2.5 cm from each sample, and stored in labelled bags at +4°C for 7 d. Raw beef colour measurements were made on samples that were matured at +4°C for 7 d. Before the colour measurements of the cooked beef, samples were stored in the refrigerator at +4°C for 7 d. The raw beef had an internal heat of 9.97 ± 3.60°C. The raw beef was cooked in a water bath (ISOLAB) at 80°C for 45 min in a fiberglass bag according to Cho *et al*. (2017). Then, the internal temperature of the cooked beef was measured to be at least 72°C. The cooked beef samples were kept in a beaker filled with water at room temperature (20 - 22°C) in a fiberglass bag for at least 15 min, until the average temperature of the beef samples was 20 - 22°C. The colour measurements of the cooked beef samples were made after they were kept in normal room temperature. The beef sample of each THB was performed in triplicate. Then, their average was evaluated for a sample. A spectrophotometer (Konica Minolta CM 5, Kaunas University of Technology, Kaunas, Lithuania) with an ID65 illuminator and 10° standard observer, with the L*, a*, and b* colour system, was used to measure the beef colour (Cho *et al*., 2017; Ardıçlı *et al.*, 2018). Colour measurement of three pieces of beef in the form of 2.5 cm cubes was made from raw and cooked MLD beef in each sample of THBs. The values of three samples of raw and cooked beefs of each THB were then averaged.

DNA isolation and PCR-RFLP genotyping

After the samples were collected from each bovine tissue, they were fragmented with tissue kits (Exiprep Tissue Genomic DNA kit, K-3225 ver. 2.0), and the genomic DNAs of the obtained products were isolated (Bioneer ExiPrepTM 16Plus innovation, Bioneer Corporation, Korea). The absorbance values of the samples (260/280 nm) were measured using an Optizen NanoQ Nanodrop micro-volume spectrophotometer (K Lab Keen Innovative Solutions, K Lab Co. Ltd., Republic of Korea). A total of 25 µL PCR amplification solution was used according to Kök and Vapur (2021). The amplification mixture consisted of 1 µL of each primer (10 pmol/µL) (Sentegen Biotech, Ankara), 12.5 µL (2X) PCR Master Mix (Dream Taq Hot Start Green, Thermo Scientific, UK), 5.5 µL purified water, and 5 µL genomic DNA (~75 ng/µL). Primer sequences used were as follows:

(i) for *LEP E2JW* = Forward: GATTCCGCCGCACCTCTC with Reverse: CCTGTGCAAGGCTGCACAGCC;

(ii) for *LEP E2FB* = Forward: ATGCGCTGTGGACCCCTGTATC with Reverse: TGGTGTCATCCTGGACCTTCC (De Oliveira *et al.*, 2013; Kök and Vapur, 2021).

(iii) For *TG C422T* = Forward: GGGATGACTACGAGTATGACTG with Reverse: GTGAAAATCTTGTGGAGGCTGTA (Shin and Chung, 2007; Kök and Vapur, 2021).

Amplification was performed by a Thermocycler (My Genie 96 Thermal Block, Bioneer Corporation, Republic of Korea), and the Touchdown PCR method was used to amplify target DNAs containing all marker variants.

The following protocol was used for the LEP E2JW: one cycle at 94°C for 2 min (denaturation); five cycles for each of the next six temperatures at 94°C for 20 s, 58°C for 20 s, 72°C for 1 min, 94°C for 20 s, 54°C for 20 s, 72°C for 1 min; and 25 cycles for each of the next three temperatures at 94°C for 20 s, 52°C for 20 s, 72°C for 1 min (annealing), and the final cycle at 72°C for 5 min (extension).

The following protocol was used for the LEP E2FB: one cycle at 94°C for 2 min (denaturation); 35 cycles for each of the next three temperatures at 94°C for 45 s, 52°C for 45 s, 72°C for 55 s (annealing); and the final cycle at 72°C for 3 min (extension).

The following protocol was used for the TG C422T: one cycle at 94°C for 5 min (denaturation); 35 cycles for each of the next three temperatures at 94°C for 1 min, 55°C for 1 min, 72°C for 1 min (annealing); and the final cycle at 72°C for 7 min (extension).

As indicated in Table 1, the PCR products (15 µL) were digested with restriction endonucleases. The enzymatic digestion reaction was incubated in a thermal cycler at 37°C for 3 h (My Genie 96 Thermal Block Bioneer Appliance, Republic of Korea). The

individual PCR-RFLP products were separated by the Advanced Analytical Fragment Analyzer capillary electrophoresis instrument (Agilent Technologies, Inc., USA), and the ProSize (Agilent Technologies, Inc.) software was used for imaging. PCR-RFLP values expressed for DNA size are approximate values owing to the nature of agarose gel electrophoresis. In the capillary electrophoresis, the size of DNA fragments was identified as exact values of ±3 base pairs. The PCR-RFLP method was performed according to Kök and Vapur (2021) (Table 1).

Table 1. Enzymes used in cutting 545 (C422T), 467 (E2JW), and 94 (E2FB) bp regions of target DNA, and 20 µL concentration content of a sample (Kök and Vapur, 2021).

Gene	Material	Content
LEP	*Kpn2I* restriction endonuclease (Anza™ 60 *Kpn2I,* Invitrogen Thermo Fisher Scientific, UK)	1 µL 1600 units (20 U/µL) or
	*BSU*15*I r*estriction endonuclease (Anza 30 *BSU15I,* Invitrogen Thermo Fisher Scientific, UK)	1 µL 1500 units (20 U/µL) or
TG	*MBOI* restriction endonuclease (Anza 55 *MBOI,* Invitrogen Thermo Fisher Scientific, UK)	1 µL 800 units (5 U/µL)
	Anza 10X white restriction buffer	2 µL
	Purified water	2 µL
	PCR product	15 µL
	Total	**20 µL**

LEP: leptin gene; TG: thyroglobulin gene; PCR: polymerase chain reaction; µL: microliter; and U/µL: Unit/microliter.

Statistical analyses

After genotypic characterisation of THBs based on three SNP variants (E2JW, E2FB, TG C422T), allele and genotypic frequencies of THBs were determined using the PopGene 32 software program according to Yeh *et al.* (2000). IBM SPSS Statistics 20.0 XLSTAT (IBM Corp., Armonk, NY, USA) demo version was used for the data analysis. Standard deviations (SD), standard errors (SE), phenotypic correlations (*r*), SW, HCW, and colour means of the phenotypic data of the samples and their relations with the marker genotypes in the SPSS program (One-way and repeated measures ANOVA, LSD, and two-tailed "*t*" test) were evaluated based on the level of significance. One-way and repeated measures ANOVA, two-tailed "*t*" test, and LSD were used to compare the means of two or more samples using the *F* distribution. Pre-slaughter (LW) and post-slaughter weights (HCW) of bulls were then measured (Kök and Vapur, 2021).

Results

In THBs, three different genotypes (AA, AT, and TT) in LEP E2JW (Figure 1), and two different genotypes (CT and TT) in LEP E2FB, were observed. Genotype frequencies were determined as 0.56, 0.38, and 0.06 for LEP E2JW, and 0.94 and 0.06 for LEP

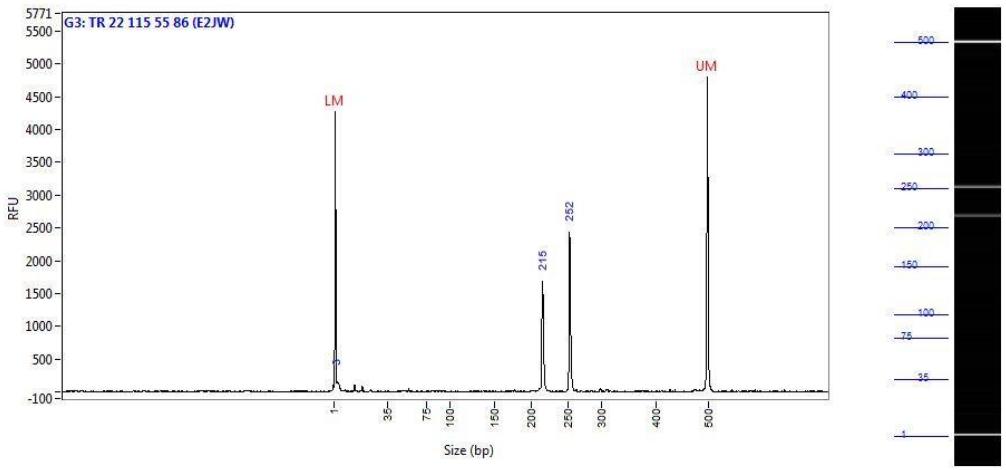

Figure 1. Electropherogram of LEP E2JW locus AA genotype.

E2FB, respectively. The A and T allele frequencies in LEP E2JW were determined as 0.75 and 0.25, and the C and T allele frequencies in LEP E2FB as 0.47 and 0.53, respectively. The TG gene was found to be monomorphic, and the C allele frequency was 1.

The frequencies of the LEP E2JW and E2FB, and TG C422T SNP variant genotypes of THBs are discussed in terms of beef colour, LW, and HCW. It was determined that the raw MLD beefs of THBs in the LEP E2JW AA marker genotype were brighter and of a more intense yellow colour than the variants in other genotypes (AT, TT) ($p < 0.05$). After the beefs were cooked, it was observed that the cooked beefs in the LEP E2JW AA and AT variant genotypes were brighter than the beefs in the TT genotype ($p < 0.05$). The a* values of the raw beefs decreased by approximately 50% in all marker genotypes after the beef was cooked (Table 2). While the differences between the a* values of the raw beefs based on the marker genotypes were insignificant ($p > 0.05$) after the MLD beefs were cooked, the redness intensity of the beefs in the AT variant genotype increased as compared to the beefs of the AA variant genotype. The redness difference was statistically significant ($p < 0.05$) (Table 2). For b* values of cooked MLD beefs, an increase of approximately 50% was observed as compared to the raw beefs of all marker variant genotypes. However, the difference in b* values of marker variant genotypes was insignificant ($p > 0.05$). It was determined that the dullest and most intensely yellow cooked beefs were in TT genotype cattle. The difference in brightness value between cooked beef in the AA and AT genotypes of the E2JW locus was statistically insignificant ($p > 0.05$). Raw beef b* value of LEP E2JW AA marker genotypes was more yellowish than that of AT and TT marker genotypes, and the difference was significant ($p < 0.05$). In general, it is a positive feature that the beefs of THBs in the LEP E2JW AA marker genotype show higher L*, a*, and b* values than the beefs of the AT and TT marker genotypes. Overall, beefs of THBs with the LEP E2JW AA marker genotype may be considered fresher for the consumer based on other genotypes (AT and TT).

LW averages of our 100 cattle samples based on the genotypes at the LEP E2JW marker locus of AA, AT, and TT were 499.76 ± 69.30, 527.55 ± 91.16, and 509 ± 71.81 kg, respectively, and the highest LW was observed in cattle with LEP E2JW AT marker genotype. LW averages of all sample cattle were 511.80 ± 79.71 kg. HCW averages were detected as 278.48 ± 41.35 kg in the LEP E2JW AA genotype, 293.45 ± 48.69 kg in the AT marker genotype, and 307.75 ± 57.02 kg in the TT genotype. The relationship between the genotypic variants determined by the LW and HCW averages of THBs, based on LEP E2JW marker locus AA, AT, and TT genotypes, was not statistically significant ($p > 0.05$). However, in terms of the bony beef yield, the best THBs were cattle in the LEP E2JW TT marker genotype (307.75 ± 57.02 kg).

Regarding the brightness of both raw and cooked types of beef (L*) of MLD beef of cattle in the genotype formed by LEP E2FB marker variants, beef in the TT marker genotype was brighter than beef in the CT marker genotype. For L* a* b* values in both raw and cooked types of beef of THBs, a statistically significant relationship was not detected in LEP E2FB CT and TT marker genotype beef ($p > 0.05$). LW averages of THBs for LEP E2FB CT and TT marker genotypes were 512.14 ± 79.78 and 506.50 ± 85.97 kg, respectively. Average HCW of THBs in the same marker genotype was 286.25 ± 45.89 kg in THBs with the CT marker genotype, and 281.00 ± 42.13 kg in cattle with the TT marker genotype. The LEP E2FB CC marker genotype was not observed in the THB samples. Based on the LEP E2FB locus CT and TT genotypes, the difference and the relationship between the mean LW and HCW of THBs were not statistically significant ($p > 0.05$).

Since the TG C422T locus was monomorphic in THB samples, no relationship could be found between beef colour (L*, a*, and b*), LW, HCW, and marker variant genotypes. Regarding colour averages of raw beef samples in the CC genotype of THBs, L* value was 40.22 ± 4.55, a* value was 10.16 ± 2.83, and b* value was 11.46 ± 2.39. For colour averages of cooked beefs, L* value was 56.62 ± 2.81, a* value was 5.83 ± 0.89, and b* value was 16.76 ± 0.75. While the raw beef samples in the TG C422T CC variant genotype were bright-red-yellow in colour, it was determined that the beef samples were bright but dull red and rather yellowish after cooking. The samples of THBs in the TG C422T CC marker genotype were monomorphic. Therefore, the colour (L*, a*, and b*) averages of raw and cooked beef samples of all THBs were determined by the TG C422T CC marker genotype bulls (Table 2). Also, it

Table 2. Effects on beef yield and colour based on LEP E2JW, LEP E2FB, and TG C422T marker genotype variants of THBs.

Phenotypic trait	**LEP E2JW**			**LEP E2FB**		**TG C422T**
($\bar{X}$ ± SD)	**AA (54)**	**AT (42)**	**TT (4)**	**CT (94)**	**TT (6)**	**CC (100)**
LW (kg)	499.76 ± 69.30	527.55 ± 91.16	509.00 ± 71.81	512.14 ± 79.78	506.50 ± 85.97	512.16 ± 79.39
HCW (kg)	278.48 ± 41.35	293.45 ± 48.69	307.75 ± 57.02	286.25 ± 45.89	281.00 ± 42.13	286.30 ± 45.40
Colour of raw beef - L* (brightness)	41.19 ± 4.43[a]	38.98 ± 4.53[b]	39.54 ± 4.91	40.10 ± 4.53	41.78 ± 5.46	40.22 ± 4.55
Colour of cooked beef - L* (brightness)	56.56 ± 2.58[a]	57.05 ± 2.67[a]	53.40 ± 5.53[b]	56.55 ± 2.87	58.13 ± 1.18	56.62 ± 2.81
Colour of raw beef - a* (redness)	10.53 ± 2.91	9.89 ± 2.75	8.87 ± 1.86	10.16 ± 2.85	10.76 ± 2.43	10.16 ± 2.83
Colour of cooked beef - a* (redness)	5.66 ± 0.83[a]	6.04 ± 0.95[b]	6.01 ± 0.81	5.84 ± 0.90	5.72 ± 0.80	5.83 ± 0.89
Colour of raw beef - b* (yellowness)	12.04 ± 2.23[a]	10.84 ± 2.44[b]	10.73 ± 2.39[b]	11.43 ± 2.39	12.31 ± 2.25	11.46 ± 2.39
Colour of cooked beef - b* (yellowness)	16.66 ± 0.79	16.85 ± 0.68	16.89 ± 0.74	16.76 ± 0.75	16.61 ± 0.58	16.76 ± 0.75

Based on ANOVA and LSD test, the genotypic differences between [a] and [b] were significant ($p < 0.05$). $\bar{X}$: arithmetic mean; SD: standard deviation; L*: brightness (0 - 100), 0 value black, 100 value white; a*: red-green (-60 to +60), lower values have more green colour, higher values have more red colour; b*: yellowish-blueness (-60 to +60), lower values have more blue colour, higher values have more yellow colour (Murray, 1995).

was determined that the average LW and HCW of the samples of THBs in the TG C422T CC marker genotype were 512.16 ± 79.39 and 286.30 ± 45.40 kg, respectively.

The percentage decrease of THBs was 55.90%, and the correlation coefficient (*r*) between LW and HCW was 0.604 ($p > 0.05$). Of the expected nine different combinations of LEP E2JW, LEP E2FB, and TG C422T triple marker genotype variants of THB which constituted our samples, only four different marker genotype combinations were observed (Table 3). The triple combinations of the LEP E2JW, LEP E2FB, and TG C422T marker genotype variants, their averages, and standard deviations for the phenotypic features of LW and HCW, and raw and cooked beef colours of Holstein bulls are given in Table 3. Based on AA/CT/CC, AT/CT/CC, AA/TT/CC, and TT/CT/CC marker variants, there was no statistically significant difference between the measured phenotypic average values ($p > 0.05$) in terms of LW and HCW, and a* and b* values of cooked beefs of THBs. However, the difference was significant ($p < 0.05$) between L*, b* of the raw beefs of THBs in the AA/CT/CC and AT/CT/CC variant genotypes, and a* values of cooked beefs. The most matte-coloured of the cooked beefs was in the TT/CT/CC variant genotype beef. The brightest colour of cooked and raw beef was in the AA/TT/CC variant genotype beef. Regarding the brightness of the cooked beefs (L*), the difference between the TT/CT/CC variant genotype and the L* values of the other observed triple variant genotypes was significant ($p < 0.05$).

Table 3. Effects on beef yield and colour based on LEP E2JW, LEP E2FB, and TG C422T marker variant combinations of THBs.

Phenotypic trait	**LEP E2JW / LEP E2FB / TG C422T genotype**			
($\bar{X}$ ± SD)	**AA/CT/CC**	**AT/CT/CC**	**AA/TT/CC**	**TT/CT/CC**
LW (kg)	498.92 ± 67.99	527.55 ± 91.16	506.50 ± 85.97	509.00 ± 71.81
HCW (kg)	278.17 ± 41.69	293.45 ± 48.69	281.00 ± 42.13	307.75 ± 57.02
Colour of raw beef - L* (brightness)	41.12 ± 4.35[a]	38.98 ± 4.53[b]	41.78 ± 5.46	39.54 ± 4.91
Colour of cooked beef - L* (brightness)	56.36 ± 2.64[a]	57.06 ± 2.67[a]	58.13 ± 1.18[a]	53.40 ± 5.53[b]
Colour of raw beef - a* (redness)	10.51 ± 2.99	9.89 ± 2.75	10.76 ± 2.43	8.87 ± 1.86
Colour of cooked beef - a* (redness)	5.65 ± 0.84[a]	6.04 ± 0.95[b]	5.73 ± 0.80[b]	6.01 ± 0.81[b]
Colour of raw beef - b* (yellowness)	12.01 ± 2.25[a]	10.85 ± 2.44[b]	12.31 ± 2.25[a]	10.73 ± 2.39 [b]
Colour of cooked beef - b* (yellowness)	16.67 ± 0.82	16.85 ± 0.68	16.61 ± 0.58	16.89 ± 0.74

Based on ANOVA, genotype differences were statistically significant ($p < 0.05$). Based on LSD, genotypic differences between [a] and [b] were significant ($p < 0.05$). $\bar{X}$: arithmetic mean; SD: standard deviation; L*: brightness (0 - 100), 0 value black, 100 value white; a*: red-green (-60 to +60), lower values have more green colour, higher values have more red colour; b*: yellowish-blueness (-60 to +60), lower values have more blue colour, higher values have more yellow colour (Murray, 1995).

Regarding the redness intensity of cooked beef (a*), the difference between the AA/CT/CC variant genotype and AT/CT/CC, AA/TT/CC, and TT/CT/CC variant genotypes was statistically significant ($p < 0.05$). The redness intensity of beefs in the AA/CT/CC variant genotype was higher than that of beef in the other triple variant genotypes ($p < 0.05$). The intensity of yellowness of raw beef (b*) in the AT/CT/CC and TT/CT/CC variant genotypes was less than the intensity of yellowness of raw beefs in the AA/CT/CC and AA/TT/CC variant genotypes ($p < 0.05$). Consequently, raw beefs of THBs carrying the LEP E2JW / E2FB and the TG C422T with A/T/C haplotype were brighter, more yellow, and redder than those with other haplotypes (A/C/C, T/T/C, T/C/C).

The phenotypic correlations (*r*) of THBs on LW, HCW, and colour traits of their beef (L*, a*, and b*) were investigated, and the results are given in Table 4. Regarding the cause of the genetic correlation, the same gene or genes are associated with affecting multiple phenotypic traits. While a phenotypic trait is desired to be improved in positive correlations, indirectly, other phenotypic trait(s) develop as positively as the positively correlated *r* effect. In phenotypic traits that are inverse, *i.e.*, negatively correlated, while a trait is tried to be genotypically developed positively, in other

Table 4. Phenotypic correlations *(r)* of THBs on LW, HCW, and colour traits of beefs (L*, a*, and b*).

Phenotypic trait	LW (kg)	HCW (kg)	Raw beef L*	Raw beef a*	Raw beef b*	Cooked beef L*	Cooked beef a*	Cooked beef b*
LW (kg)	1.00							
HCW (kg)	0.604**	1.00						
Raw beef L*	0.136	-0.084	1.00					
Raw beef a*	0.117	-0.090	0.211*	1.00				
Raw beef b*	0.042	-0.149	0.695**	0.694**	1.00			
Cooked beef L*	0.028	-0.074	0.394**	-0.063	0.213*	1.00		
Cooked beef a*	0.007	-0.092	-0.414**	-0.222*	-0.458**	-0.406**	1.00	
Cooked beef b*	-0.151	0.081	-0.432**	-0.302**	-0.278**	-0.094	0.335**	1.00

LW (live weight); and HCW (hot carcass weight). L*: brightness (0 - 100), 0 value black, 100 value white; a*: red-green (-60 to +60), lower values have more green colour, higher values have more red colour; b*: yellowish-blueness (-60 to +60), lower values have more blue colour, higher values have more yellow colour (Murray, 1995).

phenotypic features, it creates a negative development as much as the negative *r* effect. The highest correlation was found in the correlation coefficients between b* and L* (0.695) of raw beef, followed by b* and a* (0.694) of raw beef, followed by LW and HCW (0.604), and the interaction was significant ($p < 0.01$). The *r* difference between the brightness of raw beef (L*) and the average of L*, a*, and b* of cooked beefs was significant ($p < 0.01$). While there was a linear correlation (0.394) between the brightness of raw beef and the brightness of cooked beef, there was an inverse (negative) correlation between the intensities of redness (-0.414) and yellowness (-0.432) ($p < 0.01$). Still, an inverse correlation existed between a * and b* colour intensities of raw and cooked beefs. After the beef was cooked, it was observed that the redness intensity decreased, while the yellowness intensity significantly increased ($p < 0.01$). The *r* (-0.406) between L* and a* values of cooked beefs was negative, and by beef cooking, while the brightness increased, the intensity of the redness decreased. The *r*-value of L* of cooked beefs and b* of raw beefs was 0.213 ($p < 0.05$), and there was a linear *r* (0.335) correlation between a* and b* colour intensities of cooked beefs, and the differences were significant ($p < 0.01$).

Discussion

The colour of beef is under the influence of a complex process in bulls. These factors are related to the hereditary characteristics, growing and feeding conditions (extensive: in meadows and pastures; intensive: with grain-feeding in farms), and pre- and post-slaughter processes of THBs. It is possible to evaluate the effect of genetic factors on the beef colour only in cases where homogeneous environmental factors are in place.

In one study, there was a significant correlation between LEP E2FB SNP variants in Nellore (*Bos indicus*) cattle and beef colour (a*) of cattle ($p < 0.05$), and it was determined that the red colour intensity (a*) of beef in the LEP E2FB CC variant genotype was higher than that of the CT genotype (De Oliveira *et al.*, 2013). In the present work, on raw beef, the intensity of the red colour (a*) in the TT marker (10.76 ± 2.43) genotypes was more than that in the CT marker (10.16 ± 2.85) genotypes. However, after the beef was cooked, the intensity of the red colour decreased, and colour in the TT marker (5.72 ± 0.80) genotypes shifted more to green. Angus and Hereford cattle crosses, and triple cattle crosses (Angus, Hereford, and Limousin) were determined to contain more red colour intensity (a*) than beefs from Limousin and Angus cattle crosses of the same marker genotype in the LEP E2FB TT marker genotype. L* and b* values were similar in other marker genetic groups (CC and CT) of the breeds (Papaleo Mazzucco *et al.*, 2016). The brightness of raw and cooked beefs of the examined sample THBs with the LEP E2FB TT variant genotype was brighter than that of the beef of those with the CT genotype. However, in terms of cooked and raw beef brightness

(L*) of beef in LEP E2FB CT and TT variant genotypes, it was observed that differences between genotypes were not statistically significant ($p > 0.05$).

In a study of young Piedmontese bulls, TG C422T T and C allele, and a* and b* values were compared, and it was determined that there was a significant relationship between them (Ribeca *et al.*, 2014). In another study on THBs, TG C422T marker SNP was found to be polymorphic (CC and CT), and a statistically significant correlation was found between the phenotypic values of the colour parameters L* and a*, and the TG C422T variant genotypes ($p < 0.05$) (Ardıçlı *et al.*, 2018). All of the sample, THBs that we investigated were of the TG C422T CC genotype, their beefs were brighter (higher L* value), and more intensely yellow (high b* value) than Ardıçlı *et al*.'s (2018) THB beef samples in the TG C422T CC marker genotype, and lower intensity of redness (lower a* value) was observed. The differences determined in the two research groups (Ardıçlı *et al.*, 2018 and in our samples) on THB samples were thought to result from the nutritional difference of THBs. Among the cattle groups in the study by Kim *et al.* (2021), Holstein exhibited a higher brightness value as compared to Hanwoo ($p < 0.05$). Redness and yellowness did not significantly differ among the groups ($p > 0.05$). In another study, based on colour evaluation, colour scores of Holstein beef were close to each other between groups, and were slightly dark red in colour. Colour scores were nonsignificant ($p < 0.01$) (Özdemir and Yanar, 2021). No significant difference was observed between the breeds in terms of HCW based on the LEP E2FB CT and TT marker variants of Hereford, Angus, Charolais, Simmental, and Limousin breeds (Kononoff *et al.*, 2005). Relationships between LEP E2FB marker variants and HCW of Aberdeen Angus crosses (Gill *et al.*, 2009) and Argentine Brangus (5/8 Angus and 3/8 Brahman) cattle (Corva *et al.*, 2009) were investigated, and no significant relationship was found ($p > 0.05$). Based on the LEB E2FB CT and TT marker variants of Brazilian crossbred cattle, the pre-slaughter average LW of cattle was 408.65 ± 4.85 and 398.4 ± 7.09 kg, respectively, and the average HCW of cattle was 223.83 ± 3.06 and 217.61 ± 4.41 kg, respectively (De Carvalho *et al.*, 2012). Pre-slaughter LW (512.14 ± 79.78 and 506.50 ± 85.97 kg, respectively) and HCW (286.25 ± 45.89 and 281.00 ± 42.13 kg, respectively) averages of THBs of the same marker genotype were higher than in Brazilian crossbred cattle. Similarly, as in the results of previous researchers' studies of different cattle breeds and in the examination of LW and HCW in the present work, it was determined that the phenotypic differences based on the LEP E2FB marker variant genotypes were not statistically significant. In another study conducted on Western Canadian beef cattle, pre-slaughter LWs of cattle with the LEP E2FB CC, CT, and TT variant genotypes were 487.3 ± 0.6, 488.0 ± 0.5, and 484.2 ± 0.7 kg, respectively, and it was determined that the relationship between pre-slaughter LW and variant genotypes was significant. It has been reported that LW of cattle with the LEP E2FB CC and CT variant genotypes was significantly heavier than that of cattle with the LEP E2FB TT genotypes ($p < 0.05$) (Woronuk *et al.*, 2011). The LW of cattle at 17 months of age in China's native cattle (Yunling and Wenshan breed) and Simmental cattle were found to be 478.6 ± 10.4, 300.8 ± 48.9, and 505.4 ± 41.5 kg, respectively (Meng *et al.*, 2020). LW of THBs at the same age was greater than that of Chinese native and Simmental cattle.

In a study by De Carvalho *et al.* (2012) with six different cattle (Nellore, Angus, Canchim, Valdostana, Caracu, and Red Angus) crossbreeds of *B. taurus* and *B. indicus* in Brazil, pre-slaughter LW (408.06 ± 4.45 kg) and HCW (223.38 ± 2.79 kg), and again, the average of LW (442.7 ± 55.8 kg) and HCW (282.9 ± 37.9 kg) of Brahman cattle before slaughter (Casas *et al.*, 2005) was lower than the THBs. However, the average pre-slaughter LW (543.26 ± 4.54 kg) of cattle with the TG C422T CC variant genotype reported in Korean cattle (Shin and Chung, 2007) was observed to be greater than the average pre-slaughter LW (512.16 kg) of THBs which were the same variant genotype. In another study of Holstein bulls, HCW of bulls in the middle aged (MAG) and the older groups (OG) were heavier than these in the young group (YG). These differences could be considered to be from the greater final and pre-slaughter weights of the animals in MAG and OG. There were significant ($p < 0.01$) differences between HCW and pre-slaughter weights among the groups. The slaughter weights were 471.3 ± 18.5 kg in YG, 550.0 ± 16.2 kg in MAG, and 587.4 ± 20.6 kg in OG. HCW was 258.6 ± 11.8, 305.5 ± 10.3, 333.0 ± 14.3 kg, respectively (Özdemir and Yanar, 2021). The pre-slaughter LW and HCW of the THB bulls in the present work were close to the weights of the bulls in the MAG group.

Baran (2020) studied the colour values of buffalo meat, and determined the average L*, a*, and b* values as 42.66 ± 4.10, 21.66 ± 3.02, and 19.61 ± 1.31, respectively. It was understood that the L*, a*, and b* values of the raw THB beefs in the present work had a lower average than the raw buffalo meats, and the colour density of the buffalo meat was higher than that of the THB beefs. It has been reported that the L* and a* values of male buffalo meats were higher, and their b* value was lower than those of female buffaloes, but that there was no significant difference based on gender in terms of related parameters ($p > 0.05$). HCW of slaughtered buffaloes was 261.12 ± 29.71 kg in females, and 342.65 ± 26.96 kg in males, with a total average of 289.66 ± 48.77 kg in all. It was also stated that there was no significant correlation (r) relationship between the mean LW and the means of L*, a*, and b* values of all buffaloes ($p > 0.05$). While there was a negative correlation of L* ($r = -0.114$) and b* ($r = -0.104$) of buffalo meat with LW of buffaloes (Baran, 2020), there was a positive r relationship in THBs. While the r value between LW and a* of buffaloes was 0.230 (Baran, 2020), r was found to be 0.117 and lower in THBs. It is thought that as the LW of buffaloes and the LW of THBs increase, the increase in redness intensity in their meat is due to the increase in the amount of myoglobin. The L*, a*, and b* color values of raw MLD beef samples of Spanish cattle breeds were also determined as 39.66 ± 0.38, 13.37 ± 0.28, and 9.37 ± 0.19, respectively (Insausti *et al.*, 2008). While the L* values of raw beef of Spanish cattle slaughtered at an average of 470 kg LW were similar to the THBs, the raw beef a* value of Spanish cattle was higher intensely red than the raw beef of THBs. But the b* value of THBs was more intensely yellow. LWs of slaughtered Spanish cattle and male buffaloes were quite lower than THBs.

Beef colour measurements obtained from the exposed muscle of F1 Angus-Nellore cross and Nellore bulls at the same time also showed lower colourfulness in terms of yellowness and redness (Baldassini *et al.*, 2021). While a positive correlation was determined between the lumbar eye muscle (MLD) and the a* values (0.20) of Nellore (*B. indicus*) cattle, negative r correlations were observed between the marbling of beef (MS) and the a* values (-0.29) (De Oliveira *et al.*, 2013). In a study conducted on the Leptin gene of Chinese native (Yunling and Wenshan breeds) and Simmental cattle, it was determined that the correlations between beef colour (L*, a* and b* values) and pH value were significantly negatively correlated ($p < 0.01$) (Meng *et al.*, 2020). Studies on beef colour, and LW and HCW of THBs regarding the LEP E2JW marker and LEP E2JW / LEP E2FB / TG C422T triple combination marker genotype variants were not included in the discussion because they were not encountered in the literature.

Conclusion

The colour of beef is the first decisive criterion in consumer's meat preferences in whole-piece meat purchases. In beef, meat with a velvety bright cherry red colour is preferred by consumers as fresh beef. In the present work, the A allele of the LEP gene E2JW SNP marker variants had a positive contribution to the beef colour of THBs which affected beef quality. THBs are the best genotype in beef yield with bones as those of the LEP E2JW TT marker genotype (HCW = 307.75 ± 57.02 kg). However, the redness and yellowness colour intensity of the LEP E2JW TT raw beef was lower than those of the LEP E2JW AA genotype. To increase the beef yield of will-be slaughtered THBs, it is recommended be used that Turkish Holstein cattle carry the LEP E2JW TT variant genotype. But, if demanded to breed cattle for slaughter that produce brighter and more intense beef, it is recommended that cattle with the LEP E2JW AA marker genotype be used for breeding and selected at an early age by MAS method. The observed differences between genotypes were not statistically significant in terms of both cooked and raw beef brightness (L*), colour (a* and b*), LW, and HCW of the LEP E2FB CT and TT variant genotypes ($p > 0.05$). Since the TG C422T variant of THBs was monomorphic, the differences in the phenotypic features of beefs were not discussed. LEP E2JW marker variants are recommended to be used for breeding in the herd of Holstein. To generate more income from the increase in LW and HCW from THBs, the marker genotypes of THBs of the AT/CT/CC genotype should be determined by MAS method and selected for breeding in terms of LEP E2JW / E2FB / TG C422T marker loci, respectively. In addition, it is recommended that Turkish Holstein calves be placed in separate paddocks at an early age according to their marker genotypes and included in different feeding programs in fattening enterprises. It is estimated that THBs which produce bright beef will be sold more expensively than THBs of the other

marker genotypes, in places where the classification of carcasses and pieces of beef based on meat quality is implemented.

Acknowledgement

The present work was financially supported by the Trakya University Scientific Research Projects Commission (grant no.: TUBAP-2016-82).

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