Ann. Anim. Sci., Vol. 17, No. 1 (2017) 117–130 *DOI: 10.1515/aoas-2016-0045*

DE GRUYTER
OPEN

ASSOCIATION OF SINGLE NUCLEOTIDE POLYMORPHISMS IN THE *FABP4* GENE WITH CARCASS CHARACTERISTICS AND MEAT QUALITY IN HOLSTEIN BULLS*

Sena Ardicli[1], Hale Samli[1], Fazli Alpay[2], Deniz Dincel[1], Bahadir Soyudal[1], Faruk Balci[1]♦

[1]Laboratory of Genetics, Department of Genetics, Faculty of Veterinary Medicine, Uludag University, 16059, Nilufer, Bursa, Turkey
[2]Department of Animal Science, Faculty of Veterinary Medicine, Uludag University, 16059, Nilufer, Bursa, Turkey
♦Corresponding author: fbalci@uludag.edu.tr

Abstract

The aim of this study was to characterize the bovine fatty acid binding protein (*FABP4*) gene 3691G>A and 2834C>G polymorphisms and to evaluate interaction effects on the live weight, carcass characteristics and meat quality of Holstein bulls in the South Marmara region of Turkey. A total of 400 Holstein bulls grown on a private farm and slaughtered at 14–21 months of age, were randomly selected for use in this study. Initially, genotyping was performed by PCR-RFLP method and statistical analysis was carried out using least square methods of the GLM procedure. A SNP located in intron 1 (2834C>G) was associated with desirable increases in live weight, hot carcass weight and chilled carcass weight, and a SNP located in exon 3 (3691G>A) was associated with significant increases in marbling scores and first compressive stress point of the *longissimus dorsi* muscle (P<0.05). The interaction analysis of the 3691G>A and the 2834C>G polymorphisms revealed significant effects for hot carcass weight, chilled carcass weight and backfat thickness (P<0.05). There were no significant associations between the SNPs and carcass measurements. Results indicated that the *FABP4* 3691G>A and 2834C>G polymorphisms and the 3691G>A and 2834C>G interactions can be used as selection parameters in breeding programmes to improve meat yield and carcass characteristics.

Key words: *FABP4*, gene polymorphism, carcass characteristics, meat quality, Holstein

Genetic modification in the desired direction is necessary to achieve permanent and continuous progress in improving qualitative and quantitative features that are economically important in meat cattle production. For this purpose, the use of genetic markers to increase the frequency of desired genes in herds, based on selection in breeding programmes, can be used to determine properties that emerge in the

*This project was supported by the Uludag University Scientific Research Projects Centre (UAPV-20).

later stages of life, such as carcass characteristics and meat quality (Świtoński et al., 2002). Determining the expected yield and quality of meat today can be facilitated increasingly by the use of molecular studies.

In many countries, meat production can be divided into two major categories: the first one comprises companies that produce a combination of dairy or meat products, and the second comprises production from specific beef herds (Kinghorn, 1998). Cattle farms in Turkey generally produce dairy cattle and dual-purpose breeds, and the number of beef breeds is limited (Sagsöz et al., 2005). Holstein breed comprises by far the most numerous cattle breed in Turkey (Turkvet-Turkish Ministry of Food, Agriculture and Livestock Database, 2015), and therefore, improvement of the potential of Holstein meat should be considered to increase beef production.

Data concerning the quality of meat are often obtained after slaughter. Therefore, it is difficult to analyse meat quality-related properties in a living animal (Tait et al., 2014). Marker-assisted selection for genetic improvement of meat quality has very high potential (Curi et al., 2005; Gill et al., 2009). In recent years, many genes associated with growth rate, carcass weight, lean meat, marbling and texture have been specifically determined (Li et al., 2013). One of the most important characteristics of beef cattle is fat deposition and its regulation in different tissues. The amount of fat stored in different tissues directly affects the quality of the meat and plays a very important role in consumer choice (Mannen, 2011; Warner et al., 2010). Fatty acid binding protein 4 (*FABP4*) gene belongs to the family of intracellular lipid-binding proteins which has nine tissue-specific cytoplasmic FABP members (Cho et al., 2007) and has a very crucial role in regulating lipid and glucose homeostasis (Schultz et al., 2000; Tansy et al., 2003; Michal et al., 2006). Bovine *FABP4* gene, located on chromosome 14 where the functional and positional genes related to fat metabolism are localized, was suggested as a genetic factor influencing lipid catabolism (Casas et al., 2003; Fortes et al., 2009). Lipid hydrolysis and intracellular fatty acid transitions are carried out by the bovine *FABP4* gene through its interactions with hormone sensitive lipase (Tansy et al., 2003).

The *FABP4* gene encoding fatty acid binding proteins has an important function in fatty acid intake, transport and metabolism; is primarily expressed in adipose tissue; and participates in intracellular fatty acids (Gerbens et al., 1998; Matarese and Bernlohr, 1988). Because of these effects, the *FABP4* is defined as a functional and positional candidate gene for lipid synthesis, obesity (Ogino et al., 2003; Thompson, 2004), and fat distribution rate in muscle (Thaller et al., 2003; Michal et al., 2006; Barendse et al., 2009) as it is located within a quantitiative traits loci (QTL) region (Cattle QTL Database, 2009). To the best of our knowledge, there is no information about the *FABP4* 3691G>A and 2834C>G polymorphisms in Holsteins.

Determination of possible associations between gene polymorphisms and carcass characteristics may be useful in improving meat yield and quality. Therefore, the aim of this study was to investigate the bovine *FABP4* gene 3691G>A and 2834C>G polymorphisms and interaction effects that are associated with live weight, carcass characteristics and meat quality in Holstein bulls in the South Marmara region of Turkey.

Material and methods

Animals

A total of 400 Holstein bulls that were grown on a private farm in the South Marmara region and slaughtered at 14–21 months of age were selected randomly for the collection of genotype and carcass traits. All animals (Turkish origin) 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 (05.09.2012/74). Animals were fasted for 12 hours, and pre-slaughter body weights were recorded by precision balance to within 100 grams. Previous to slaughter, stunning method was applied with a captive bolt stunner.

Carcass characteristics

For the study, live weight (LW), hot carcass weight (HCW) and chilled carcass weight (CCW) of all animals were recorded. CCW was measured after 24 hours at 4°C. The dressing percentage (DP) was calculated based on HCW. After slaughter, carcass length (CL), rump length (RL), rump width (RW), chest width (CW) and inner chest depth (ICD) dimensions were measured with a caliper, cane and ruler. CL is the distance from the os pubis to the tip of the 1st rib, and RL is the distance from the os calcaneus to the median point of the os pubis. RW was measured (n=365) from the rump circumference starting from the point opposite the meat section to the line connecting the center of the os pubis and the os calcaneus. CW is the outer side of the half carcass section from the 6th rib tip to the 6th vertebrae, and ICD was measured from the 6th rib tip to the 6th vertebrae on the inner side of the half carcass section. Backfat thickness (BFT) was measured from the lateral side of the *musculus longissimus dorsi* corresponding to ¾ of the 12th rib alignment (Sagsöz et al., 2005). The 12th rib surface of the *musculus longissimus dorsi* (MLD) area was copied to acetate paper after the 12th intercostal region had been cut, and the surface area was calculated by using a planimeter (Ushikata X-Plan 380d III).

Meat quality traits

Meat quality traits evaluated were marbling score (MS), pH, colour (L*, a* and b*) parameters, Warner-Bratzler shear force (WBSF), first compressive stress point (FCS), cooking loss (CL) and water holding capacity (WHC) in the present study.

The marbling score was evaluated by using a scale (visual evaluation) according to the USDA standards to represent 9 degrees of marbling (practically devoid, traces, slight, small, modest, moderate, slightly abundant, moderately abundant, abundant) for determining the fat distribution between the muscle fibres (Hilton et al., 1998). The pH was measured in the *musculus longissimus dorsi* between the 12th and 13th ribs with an electronic pH meter (Testo 205 pH meter). Meat colour measurements were performed with a spectrocolorimeter (Konica Minolta CM508d) that uses the coordinate system of L* (lightness), a* (redness) and b* (yellowness). The D65 light source was selected. The device was calibrated with the standard white plate before measurements. The spectrocolorimeter was set to make 3 measurements and take their average. Measurements were repeated 3 times on the meat samples of *musculus*

longissimus dorsi that had been chilled for 24 hours at 4°C after slaughter, and the average of these measurements was evaluated as the final value.

Five-centimetre-thick meat samples from the *musculus longissimus dorsi* (12th-13th intercostal area) were taken from 50 bulls randomly selected to determine the parameters of tenderness, CL and WHC. The samples derived from *musculus longissimus dorsi* vacuum-packed and aged for 8–10 days at 2°C. This procedure of aging was only used in order to provide a wider range in tenderness. Thus, ageing length was not considered in the statistical analysis (Destefanis et al., 2008). At the end of the pre-established ageing period, each sample was stored at –20°C until utilization.

Initially, the meat samples were thawed in a cooler at 2°C for 24 hours. In order to measure CL (%), meat samples were firstly weighed, and then cooked in a 75°C water bath for 60 minutes and cooled for 60 minutes under water. After being removed from their package and dried, the samples were weighed. CL (%) was evaluated from the before and after cooking weights: the formula of (weight before cooking – weight after cooking)/weight before cooking × 100 was used (Juszczuk-Kubiak et al., 2004). After the measurement of cooking loss, cooked samples were used to determine shear force value.

Shear force values of sub-samples were determined using an Instron Universal Testing Machine (Model 3343) equipped with a Warner-Bratzler (WB) shear force apparatus. The force applied to the meat was set to 50 kg, and blade speed was adjusted to 150 mm/min. To determine the peak shear force, 6 meat samples, 3 cm long and with 1×1 cm sections, were prepared, the maximum force was applied during cutting of the blade (kg/cm^2), and the force × time graphics were recorded. WBSF value and FCS of each sample were determined by taking the average of the measurements obtained from the 6 samples (Thompson, 2004). A 'modified Grau and Hamm' method was applied to determine the WHC (Grau and Hamm, 1953).

Genomic DNA isolation

For this study, 4 ml blood samples were collected in EDTA tubes from the vena jugularis of each of the bulls. The blood samples were stored at 4°C and sent to Uludag University Faculty of Veterinary Medicine, Laboratory of Genetics. DNA isolation was performed by a phenol-chloroform method. The amount and purity of the DNA samples was measured with a spectrophotometer (NanoDrop 2000c). DNA samples were stored at –80°C until the PCR-RFLP was performed.

PCR-RFLP analysis

In this study, the 3691G>A polymorphism located in exon 3 and the 2834C>G polymorphism located in intron 1 of the *FABP4* gene were examined by the PCR-RFLP method. A nucleotide position at 3691 revealed an amino acid substitution (Valine to Methionine) in *FABP4* 3691G>A SNP. PCR was performed with appropriate primers on the obtained DNA samples as described by Shin et al. (2012).

(a) *FABP4* 3691G>A (565 bp- identified with *Nla III*)
F: 5' ACCCCTATGATGCTATTCCACA 3'
R: 5' ATACGGTTCACATTGAGAGGGA 3'

(b) *FABP4* 2834C>G (590 bp- identified with *Hpy188*I)
F: 5' GCTGCTCTCTCATGGTTAAGATGG 3'
R: 5' CCTTGACTTTCCTGTCATCTGG 3'

The PCR amplification was performed in a total volume of 50 µl containing 33.5 µl dH_2O, 5 µl 10× Buffer, 5 µl $MgSO_4$, 1 µl dNTPs (2.5 mM), 2.5 U Taq DNA polymerase (Biomatik, A1003–500U, 5U/µl), 1 µl (0.025 µM) of each primer, and 3 µl of the DNA sample at a concentration of 100 ng/µl. The DNA amplification reactions were performed in a Thermal Cycler (Palm Cycler GC1-96, Corbett Reseach) with thermal conditions as follows: 95°C for 4 min, followed by 35 cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 1.5 min, followed by a final extension step at 72°C for 5 min. After amplification, 15 µl of the PCR product with each SNP was digested in 15 units of the corresponding restriction enzyme. These reactions were incubated at 37°C for 16 hours. The digestion products were then electrophoresed in 3% agarose gel (Sigma Aldrich, Steinheim, Germany) at 85–90 v for 1 hour after incubation and photographed with a gel imaging system (DNr-Minilumi).

Statistical analysis

The Hardy-Weinberg equilibrium (HWE) was tested for all alleles by using the Court Lab HWE Calculator. The effects of single and combined genotypes on the traits studied were analysed by the least-squares method as applied in a general linear model (GLM) procedure of Minitab (MINITAB®, USA, v17.1.0) according to the following listed statistical models.

The model for the slaughter and carcass traits is as follows:

$$Y_{ijklm} = \mu + S_i + W_j + AG_k + CG_l + I_{kl} + e_{ijklmn}$$

where:

Y_{ijklm} = the studied traits;
μ = the overall mean;
S_i = the fixed effect of season at the slaughter (i = winter,…, summer);
W_j = the fixed effect of age at slaughter (j = ≤14, 15, …, 20, ≤21);
AG_k = the fixed effect of the *FABP4* genotype for the 3691 G>A (k = GG, GA, AA);
CG_l = the fixed effect of the *FABP4* genotype for the 2834C>G (k = CC, CG, GG);
I_{kl} = the fixed effect of the interaction of the 2834G>A and the 2834C>G genotypes;
e_{ijklmn} = the random residual effect.

The model for the meat quality traits is as follows:

$$Y_{ijklm} = \mu + S_i + W_j + AG_k + CG_l + \beta (X_{im} - X_m) + e_{ijklmn}$$

where:

Y_{ijklm} = the studied traits;

μ = the overall mean;

S_i = the fixed effect of season at the slaughter (i = winter,…, summer);

W_j = the fixed effect of live body weight at slaughter (j = <429, 430-479, 480-529, 530<);

AG_k = the fixed effect of the *FABP4* genotype for 3691G>A (k = GG, GA, AA);

CG_l = the fixed effect of the *FABP4* genotype for 2834C>G (k = CC, CG, GG);

$\beta\ (X_{im} - X_m)$ = the regression effect of slaughter age on the WBSF, FCS, CL or WHC (m=381 to 786);

e_{ijklmn} = the random residual effect.

When significant associations were identified, the mean values for each genotype were contrasted using Tukey's test.

Results

Polymorphism in the *FABP4* gene

We investigated two SNP located in exon 3 and intron 1 of the *FABP4* gene, as reported by Shin et al. (2012). The gene frequencies, population genetic indices (He, PIC) and compatibility with the Hardy-Weinberg equilibrium are shown in Table 1. Results show that the population was determined not to be compatible for either genotype in the Hardy-Weinberg equilibrium (P<0.001). The 3691G>A showed the low frequency of allele A (27%) while the 2834C>G showed the low frequency of allele C (39%) in the present study. For the polymorphism of 3691G>A, even though the GG genotype frequency is close to the GA frequency, the AA genotype frequency is rather low compared to the other two genotypes (4%). Besides, the frequency of the GC genotype in the 2834C>G polymorphism is high compared to that of the other two genotypes, the frequency of the CC genotype is relatively low (9%).

Table 1. *FABP4* 3691G>A and 2834C>G gene frequencies, population genetic indices and HWE test for genotypes in Holstein population

	3691 G>A Genotype			2834 C>G Genotype		
	AA	GA	GG	CC	GC	GG
N	16	183	201	36	243	121
%	4.00	45.75	50.25	9.00	60.75	30.25
MAF		0.27			0.39	
He		0.3942			0.4758	
PIC		0.3165			0.3626	
χ2(HWE)*		10.75			29.69	

χ^2(HWE): Hardy-Weinberg equilibrium; χ^2 value; *P<0.001.

N: number of experimental bulls; MAF: minor allele frequency; He: heterozygosity; PIC: polymorphism information content; HWE: Hardy-Weinberg equilibrium.

Table 2. Least square means for *FABP4* 3691G>A and 2834C>G genotype effects on carcass traits and meat quality (N=400)

Genotype	N	LW (kg)	HCW (kg)	CCW (kg)	DP (%)	BFT (mm)	MLD (cm^2)	MS (1–9)	pH
3691 G>A									
AA	16	481.0	263.7	259.4	54.65	3.19	94.92	1.59 c	5.55
GA	183	498.9	267.8	263.4	53.67	2.98	100.37	2.22 b	5.58
GG	201	479.7	257.3	253.0	53.55	2.83	100.68	3.19 a	5.58
		NS	NS	NS	NS	NS	NS	P<0.05	NS
2834 C>G									
CC	36	501.6 a	273.9 a	269.6 a	54.52	3.20	97.14	2.45	5.54
GC	243	491.6 a	263.8 a	259.4 ab	53.62	3.06	99.99	2.35	5.58
GG	121	466.4 b	251.0 b	246.7 b	53.75	2.74	98.83	2.21	5.60
		P<0.05	P<0.05	P<0.05	NS	NS	NS	NS	NS
Genotype									
AACC	5	509.5	286.3 a	282.1 a	56.02	3.74 a	88.94	1.85	5.51
AAGC	6	504.1	272.9 ab	268.2 ab	54.03	3.53 a	98.61	1.63	5.54
AAGG	5	429.3	231.8 c	227.8 c	53.91	2.31 b	97.20	1.29	5.61
GACC	8	511.8	276.6 ab	272.1 ab	54.03	3.10 ab	100.11	2.24	5.57
GAGC	117	488.8	260.9 bc	256.6 bc	53.46	2.80 ab	101.65	2.17	5.58
GAGG	58	496.0	265.8 ab	261.4 ab	53.53	3.06 ab	99.36	2.26	5.59
GGCC	23	483.5	258.9 bc	254.7 bc	53.50	2.76 b	102.38	3.26	5.54
GGGC	120	481.8	257.5 bc	253.2 bc	53.36	2.86 b	99.72	3.24	5.61
GGGG	58	473.8	255.3 bc	251.0 bc	53.80	2.86 b	99.95	3.08	5.58
		NS	P<0.05	P<0.05	NS	P<0.05	NS	NS	NS
PSE		8.26	4.69	4.64	0.32	0.14	2.03	0.10	0.02

N: number of animals; LW: live weight; HCW: hot carcass weight; CCW: chilled carcass weight; DP: dressing percentage; BFT: backfat thickness; MLD: *Musculus longissimus dorsi* area; MS: marbling score; PSE: pooled standard error; NS: non-significant.

a, b, c – different letters within a column indicate significant difference (P<0.05).

Table 3. Least square means for *FABP4* 3691G>A and 2834C>G genotype effects on carcass measurement and meat colour (N=400)

Genotype	N	CL (cm)	RL (cm)	RW** (cm)	CW (cm)	ICD (cm)	L*	a*	b*
3691 G>A									
AA	16	140.9	62.86	101.13	82.07	59.92	33.41	11.04	9.52
GA	183	140.8	63.75	100.71	81.76	59.90	34.84	11.46	9.11
GG	201	140.1	63.86	100.50	81.12	59.27	34.60	10.78	9.45
		NS	NS	NS	NS	NS	NS	NS	NS
2834 C>G									
CC	36	140.8	62.93	100.95	82.12	59.99	32.90	10.08	9.47
GC	243	140.6	63.97	100.59	81.63	59.49	35.36	11.96	9.79
GG	121	140.4	63.57	100.80	81.20	59.61	34.60	11.23	8.82
		NS	NS	NS	NS	NS	NS	NS	NS
Genotype									
AACC	5	141.1	61.60	-	82.97	60.15	29.79	8.07	9.98
AAGC	6	141.0	64.26	-	82.69	60.07	36.85	13.63	10.13
AAGG	5	140.7	62.71	-	80.54	59.55	33.60	11.42	8.47
GACC	8	141.0	63.89	-	81.74	60.26	34.63	10.90	9.48
GAGC	117	140.6	63.61	-	81.36	59.46	34.67	11.41	9.41
GAGG	58	140.6	63.73	-	82.18	59.97	35.24	12.06	8.45
GGCC	23	140.4	63.28	-	81.66	59.56	34.28	11.26	8.95
GGGC	120	140.2	64.03	-	80.83	58.95	34.56	10.85	9.85
GGGG	58	139.8	64.28	-	80.86	59.30	34.96	10.22	9.55
		NS	NS	-	NS	NS	NS	NS	NS
PSE		0.46	0.26	0.27	0.46	0.36	0.64	0.45	0.44

N: number of animals; CL: carcass length; RL: rump length; RW: rump width; CW: chest width; ICD: inner chest depth; L* (lightness), a* (redness), b* (yellowness): meat colour parameters; PSE: pooled standard error; NS: non-significant.

**Rump width (RW) was measured in 365 animals. Genotypic distribution was not adequate to perform interaction analysis.

In this study, the minor allele frequencies for 3691G>A and 2834C>G markers were 0.27 and 0.39 respectively (Table 1). According to the classification reported by Menezes et al. (2006), which considered a polymorphic locus when the frequency of the most common allele was lower than 0.95, both markers in the present study were polymorphic. The 2834C>G marker indicated a higher value of heterozygosity (He) compared with 3691G>A polymorphism. The polymorphism information content (PIC) values were 0.3165 for 3691G>A and 0.3626 for 2834C>G. According to the classification reported by Botstein et al. (1980), a marker with a PIC value higher than 0.5 is considered to be very informative, whereas values between 0.25 and 0.5 are mildly informative, and values lower than 0.25 are not informative. The PIC values found for 3691G>A and 2834C>G, indicated that the markers were mildly informative in the present study.

Relationship between the *FABP4* genotypes and carcass traits and meat quality

The results of the association analysis between polymorphisms and carcass traits and meat quality parameters are shown in Tables 2–4. Table 2 shows least square means of LW, HCW, CCW, DP, BFT, MLD area, MS and carcass pH for *FABP4* 3691G>A and 2834C>G genotypes. The results indicated that bulls with the GG genotype for 3691G>A had a significantly greater MS ($P<0.05$). The polymorphism 2834C>G was significantly associated with the following traits: LW, HCW and CCW (Table 2). In addition, significant associations ($P<0.05$) were found between *FABP4* 3691G>A / 2834C>G interaction and HCW, CCW and BFT.

Table 4. Least square means for *FABP4* 3691G>A and 2834C>G genotype effects on WBSF, FCS, CL and WHC (N=50)

Genotype	N	WBSF (kg/cm^2)	FCS (kgf/cm^2)	CL (%)	WHC (%)
3691 G>A					
AA	5	10.36	110.36 a	34.80	15.25
GA	21	4.68	45.31 b	24.73	13.08
GG	24	5.44	53.78 b	24.77	12.52
		NS	P<0.05	NS	NS
2834 C>G					
CC	9	6.80	68.85	27.09	13.48
GC	25	6.51	67.27	28.52	13.81
GG	16	7.17	73.33	28.69	13.57
		NS	NS	NS	NS
PSE		4.30	44.18	11.28	4.94

N: number of animals; WBSF: Warner-Bratzler shear force; FCS: first compressive stress; CL: cooking loss; WHC: water holding capacity; PSE: pooled standard error; NS: non-significant.

a, b – different letters within a column indicate significant difference ($P<0.05$).

Table 3 shows that non-significant differences among *FABP4* genotypes were found for carcass measurement and meat colour parameters (L*, a* and b*). Interaction analysis revealed similar results, and these were found to be statistically non-significant (P>0.05). However, the association between *FABP4* 3691G>A/2834C>G and a* value tends to be significant (P=0.059).

Least square means, shown in Table 4, indicated that bulls with AA genotype for *FABP4* 3691G>A had a significantly greater FCS in the *longissimus dorsi* muscle (P<0.05), while no difference was observed in WBSF. Besides, the association of the *FABP4* genotypes with CL (%) and WHC was found to be non-significant.

Discussion

Polymorphism in the *FABP4* gene

The present results showed a lower frequency of allele A (27%) of the 3691G>A polymorphism in Holstein breed, resulting in low genetic variabilities of He and PIC compared with the 2834C>G polymorphism. A similar frequency (21%) has been reported by Shin et al. (2012) in Hanwoo cattle. With respect to the 2834C>G polymorphism, allele G was more frequent (61%). However, Shin et al. (2012) reported that the frequency of allele C was higher (59%) than that of allele G. Nevertheless, the allele and genotype frequencies are known to vary between breeds and even between different populations of the same breed. Evaluating the existence of epistasis, genetic linkage and pleiotropy is important to consider different combinations of the polymorphisms (Carvalho et al., 2012).

Relationship between the *FABP4* genotypes and carcass traits and meat quality

Our results indicated that polymorphism within *FABP4* gene may be associated with meat yield and quality. In the association analyses, the polymorphism 2834C>G significantly affected the LW, HCW and CCW (P<0.05). Among the factors considered, animals with CC genotype had higher rates of corresponding traits compared to the other two genotypes in the present study. Conversely, Shin et al. (2012) found no association of 2834C>G with CW in Hanwoo cattle. Breed type and the production environment of the animals determine the slaughter weight and carcass traits (Sanudo et al., 2004). Besides, studies on the association of the *FABP4* gene with meat and carcass yield in Holstein are insufficient (Narukami et al., 2011). The 3691G>A did not directly influence the carcass weights in the present study. However, the *FABP4* 3691G>A / 2834C>G interaction analysis showed that animals with AACC and GACC genotypes were significantly different compared to those with other genotypes (P<0.05). These results indicated that evaluation of the CC genotype in the 2834C>G polymorphism and the GA genotypes in the 3691G>A polymorphism may provide valuable benefits for LW, HCW and CCW. Further studies investigating these markers need to be performed before using them in marker-assisted selection.

Our results suggested that the individual effects of these SNPs were not statistically significant for BFT. Shin et al. (2012) also found no significant association between the *FABP4* polymorphisms and BFT in Hanwoo cattle. Interestingly, the combined effects of the 3691G>A and the 2834C>G polymorphisms indicated a statistically significant interaction ($P<0.05$) in our study. Animals with the AAGG genotype exhibited both low fat and low body weight. Moreover, the decrease in body weight in animals with the AAGG genotype might be caused by the low fat storage capacity of these animals, as these animals also had lower values for carcass length, rump height and width, chest width, and inner chest depth compared to the other genotypes. These results demonstrated that a portion of the differences in live weight and carcass weight is dependent on the overall fat content of the individual. Michal et al. (2006) reported significant associations of the *FABP4* g.7516 G>C polymorphism with subcutaneous fat depth and deposition in F2 Wagyu × Limousin cattle. SNP g.7516 G>C contained a restriction site of *Msp A1*I that was 20 bp away from the identified SNP of the *FABP4* 3691G>A in our study. In the literature there is limited information about the association of 3691G>A and 2834C>G markers with carcass characteristics and our results suggest that focusing on this genomic region may be useful in improving these traits.

Marbling is an important meat quality trait in that it contributes directly to the value of meat on customers choice, but information about the genetic basis of this trait is limited (Harper et al., 2004). *FABP4* is known as an important gene regulating the fat content among muscle fibres, thus influencing the marble appearance (Maharani et al., 2012). Therefore, information of the SNPs in *FABP4* is very important to provide clues about the genetic variations of meat quality which are determining factors in cattle industry and commercial lines. In the present study, 3691G>A polymorphism of the *FABP4* gene was significantly associated with MS ($P<0.05$). The statistical analysis revealed that the effects of the GG genotypes in the 3691G>A SNP were significantly greater than the AA and heterozygote genotypes for MS. The results may imply that animals with allele G have a great impact to commercial markets. Accordingly, the present study supports the findings of Shin et al. (2012) and suggests 3691G>A locus as an important genetic marker in breeding programmes for the improvement of meat quality traits. In this respect, evaluations of the associations between bovine *FABP4* and meat quality traits may provide tremendous economic benefits.

The statistical analysis revealed that the effects by the AA genotype for the 3691G>A SNP were significantly associated with an increase in FCS ($P<0.05$), but showed no detectable effects of *FABP4* polymorphisms on WBSF. Studies performed with larger populations may be needed to confirm these associations.

A number of potential candidate genes containing genetic variants have been evaluated to identify novel associations with economically important traits. It is also important to consider how genotypic interactions influence the phenotypic variations. The functional significance of the genes with the substitution of amino acids by SNPs may change protein structures, resulting in altered mRNA translation and protein synthesis mechanisms. However, presence or absence of an intron can dictate the mechanism of mRNA export. The export pathway used might depend

on alternate promoter usage and could influence gene expression on several levels. Therefore evaluating the exon-intron mechanisms plays a key role to understand the genetic background of quantitative traits (Le Hir et al., 2003). Our results indicate that the interactions between the 3691G>A (exon 3) and 2834C>G (intron 1) polymorphisms for HCW, CCW and BFT may be evaluated as a selection parameter. Thus, the results suggest that significant economic benefits can be achieved from selecting for these SNPs.

Conclusions

Our study showed that *FABP4* gene polymorphisms may have potential for meat yield and quality. Besides, this study is the only report investigating the *FABP4* 3691G>A and 2834C>G polymorphisms in Holstein populations to evaluate the carcass characteristics and meat quality. Focusing on these possible associations and characterization of *FABP4* is highly relevant for elucidating the influence of this genomic region on observed carcass characteristics and meat quality traits such as weight gain, fat deposition, marbling score and body-fat mass in marker-assisted selection programmes. Moreover, the results could therefore be indicative for future studies on lipid and glucose homeostasis in livestock.

Acknowledgments

The authors gratefully thank 'Banvit Incorporated Company' for help in managing the animals and collecting meat samples. This research was supported by the Uludag University Scientific Research Projects Centre (Grant No. UAPV-2011/20).

References

Barendse W., Bunch R.J., Thomas M.B., Harrison B.E. (2009). A splice site single nucleotide polymorphisms of the fatty acid binding protein 4 gene appears to be associated with intramuscular fat deposition in longissimus muscle in Australian cattle. Anim. Genet., 40: 70–73.

Botstein D., White R.L., Skolnick M., Davis R.W. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Genet., 32: 314–331.

Carvalho T.D., Siqueira F., Torres Junior R.A.A., Medeiros S.R. (2012). Association of polymorphisms in the leptin and thyroglobulin genes with meat quality and carcass traits in beef cattle. R. Bras. Zootec., 41: 2162–2168.

Casas E., Shackelford S.D., Keele J.W., Koohmaraie M. (2003). Detection of quantitative trait loci for growth and carcass composition in cattle. J. Anim. Sci., 81: 76–83.

Cattle QTL Database (2009). http://www.animalgenome. org/cgi-bin/QTLdb/BT/index

Cho S., Park T.S., Yoon D., Cheong H.S., Park B.L., Lee H.W., Han C.S. (2008). Identification of genetic polymorphisms in FABP3 and FABP4 and putative association with back fat thickness in Korean native cattle. BMB Rep., 41: 29–33.

Curi R.A., Palmieri D.A., Suguisawa L., Ferraz A.L.J., Oliveira H.N., Furlan L.R., Silveira A.C., Lopes C.R. (2005). Effects of GHR gene polymorphisms on growth and carcass traits in Zebu and crossbred beef cattle. Anim. Genet., 36: 58–62.

Destefanis G., Brugiapaglia A., Barge M.T., Dal Molin E. (2008). Relationship between beef consumer tenderness perception and Warner–Bratzler shear force. Meat Sci., 78: 153–156.

Fortes M.R.S., Curi R.A., Chardulo L.A.L., Silveira A.C., Assumpção M.E.O.D.,

Visintin J.A., Oliveira H.N. (2009). Bovine gene polymorphisms related to fat deposition and meat tenderness. Genet. Mol. Biol., 32: 75–82.
Gerbens A., Jansen A., Van Erp J.M., Harders F., Meuwissen T.H.E., Rettenberger G. (1998). The adipocyte fatty acid-binding protein locus: characterisation and association with intramuscular fat content in pigs. Mamm. Genome, 9: 22–26.
Gill J.L., Bishop S., McCorquodale C., Williams J.L., Wiener P. (2009). Association of selected SNP with carcass and taste panel assessed meat quality traits in a commercial population of Aberdeen Angus-sired beef cattle. Genet. Sel. Evol., 41, p. 36.
Grau R., Hamm G. (1953). Eine einfache Methode zur Bestimmung der Wasserbindung in Muskel. Naturwissenschaften, 40: 277–259.
Harper G.S., Pethick D.W. (2004). How might marbling begin? Aust. J. Exp. Agric., 44: 653–662.
Hilton G.G., Tatum J.D., Williams S.E., Belk K.E., Williams F.L., Wise J.W., Smith G.C. (1998). An evaluation of current and alternative systems for quality grading carcasses of mature slaughter cows. J. Anim. Sci., 76: 2094–2013.
Juszczuk-Kubiak E., Rosochacki S.J., Wicinska K., Szreder T., Sakowski T. (2004). A novel RFLP/AluI polymorphism of the bovine calpastatin (CAST) gene and its association with selected traits of beef. Anim. Sci. Pap. Rep., 22: 195–204.
Kinghorn B.P. (1998). Future developments in animal breeding. J. Anim. Sci., 28: 27–32.
Le Hir H., Nott A., Moore M.J. (2003). How introns influence and enhance eukaryotic gene expression. Trends Biochem. Sci., 28: 215–220.
Li X., Ekerljung M., Lundstrom K., Lunden A. (2013). Association of polymorphisms at DGAT1, Leptin, SCD1, CAPN1 and CAST genes with color, marbling and water holding capacity in meat from beef cattle populations in Sweden. Meat Sci., 94: 153–158.
Maharani D., Jung Y., Jung W.Y., Jo C., Ryoo S.H., Lee S.H., Yeon S.H., Lee J.H. (2012). Association of five candidate genes with fatty acid composition in Korean cattle. Mol. Biol. Rep., 39: 13–21.
Mannen H. (2011). Identification and utilization of genes associated with beef qualities. Anim. Sci. J., 82: 1–7.
Matarese V., Bernlohr D.A. (1988). Purification of murine adipocyte lipid-binding protein: characterisation as a fatty acid-and retinoic acid-binding protein. J. Biol. Chem., 263: 544–551.
Menezes M.P.C., Martinez A.M., Riberio M.N., Pimenta Filho E.C., Bermejo J.V.D. (2006). Genetic characterization of Brazilian native breeds of goats using 27 markers microsatellites. R. Bras. Zootec., 35: 1336–1341.
Michal J.J., Zhang Z.W., Gaskins C.T., Jiang Z. (2006). The bovine fatty acid binding protein 4 gene is significantly associated with marbling and subcutaneous fat depth in Wagyu × Limousin F2 crosses. Anim. Genet., 37: 400–402.
Narukami T., Sasazaki S., Oyama K., Nogi T., Taniguchi M., Mannen H. (2011). Effect of DNA polymorphisms related to fatty acid composition in adipose tissue of Holstein cattle. Anim. Sci. J., 82: 406–411.
Ogino T., Moraleio D.H., Kose H., Yamada T., Matsumoto K. (2003). Serum leptin concentration is linked to chromosome 2 and 6 in the OLETF rat, an animal model of type 2 diabetes with mild obesity. Mamm. Genome, 14: 39–44.
Sagsöz Y., Coban O., Lacin E., Sabuncuoglu N., Yildiz A. (2005). Fattening performance and carcass features of Brown Swiss and Charolais × Brown Swiss calves (in Turkish). Atatürk Üniv. Ziraat Fak. Derg., 36: 163–169.
Sanudo C., Macie E.S., Olleta J.L., Villarroel M., Panea B., Alberti P. (2004). The effects of slaughter weight, breed type and ageing time on beef meat quality using two different texture devices. Meat Sci., 66: 925–932.
Schultz J.R., Tu H., Luk A., Repa J.J., Medina J.C., Li L., Schwendner S., Wang S., Thoolen M., Mangelsdorf D.J., Lustig K.D., Shan B. (2000). Role of LXRs in control of lipogenesis. Genes Dev., 14: 31–38.
Shin S.C., Heo J.H., Chung E.R. (2012). Genetic variants of the FABP4 gene are associated with marbling scores and meat quality grades in Hanwoo (Korean cattle). Mol. Biol. Rep., 39: 23–30.
Świtoński M. (2002). Molecular genetics in beef cattle breeding – a review. Anim. Sci. Pap. Rep., 20: 7–18.

Tait R.G., Shackelford S.D., Wheeler T.L., King D.A., Keele J.W., Casas E., Smith T.P., Bennett G.L. (2014). CAPN1, CAST, and DGAT1 genetic effects on preweaning performance, carcass quality traits and residual variance of tenderness in a beef cattle population selected for haplotype and allele equalization. J. Anim. Sci., 92: 82–93.

Tansy J.T., Huml A.M., Vogt R., Davis K.E., Jones J.M., Fraser K.A., Brasaemle D.L., Kimmel A.R., Londos C. (2003). Functional studies on native and mutated forms of Perilipins. A role in protein kinase A-mediated lipolysis of triacylglycerols in Chınese hamster ovary cells. J. Biol. Chem., 278: 1–6.

Thaller G., Kuhn C., Winter A., Ewald G., Bellmann O., Wegner J., Zuhlke H., Fries R. (2003). DGAT1, a new positional and functional candidate gene for intramuscular fat deposition in cattle. Anim. Genet., 34: 354–357.

Thompson J.M. (2004). The effects of marbling on flavour and juiciness scores of cooked beef, after adjusting to a constant tenderness. Aust. J. Exp. Agric., 44: 645–652.

Turkvet-Turkish Ministry of Food, Agriculture and Livestock Database. (2015). www.turkvet.gov.tr

Warner R.D., Greenwood P.L., Pethick D.W., Ferguson D.M. (2010). Genetic and environmental effects on meat quality. Meat Sci., 86: 171–183.

Received: 24 III 2016
Accepted: 1 VII 2016