The association of *CAPN1, CAST, SCD,* and *FASN* polymorphisms with beef quality traits in commercial crossbred cattle in the Czech Republic

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ABSTRACT: Genetic variability of four polymorphisms (*CAPN1, CAST, SCD,* and *FASN*) was evaluated and the relationships between them and the beef quality traits (tenderness and fatty acid composition) in Czech crossbred cattle population were assessed. Totally 331 animals were genotyped using multiplex PCR-RFLP. For the *CAST* NM174003.2:c.155C>T the *CC* genotype was associated with higher Warner-Bratzler shear force (*CC* > *CT*, $P < 0.001$; *CC* > *TT*, $P < 0.05$) and our results (*CC* > *TT* > *CT*) suggest a possible effect of over-dominance. The *CAPN1* AF252504.2:c.947G>C did not significantly influence the beef tenderness (strongly influenced by misbalance between the genotype frequencies) suggesting the breed specific effect of this marker. Our association study of the *SCD* AB075020:c.878T>C polymorphism revealed a positive effect of allele *C* on myristoleic acid content (*CC* > *CT* > *TT*, $P < 0.01$). The significant influence of *FASN* AF285607:g.17924A>G on fatty acid profile was confirmed in the content of myristoleic acid (*AG* > *GG*, $P < 0.05$), palmitoleic acid (*AA* > *AG* > *GG*, $P < 0.05$, $P < 0.001$), stearic acid (*AA* > *GG*, $P < 0.05$), myristic acid (*AG* > *GG*, $P < 0.05$), and palmitic acid (*AG* > *GG*, $P < 0.05$). Thus in our population the SNPs of *FASN* and *SCD* appeared to be useful markers for selection of animals according to the fatty acid profile as well as SNP of *CAST* for beef tenderness.

Keywords: candidate genes; tenderness; fatty acids composition

There has been a significant interest in genetic selection to reduce problems with beef tenderness variation, which has a significant impact on consumers' satisfaction (Page et al., 2002). The calpain proteolytic system – the micromolar calcium-activated neutral protease μ-calpain encoded by the *CAPN1* gene, and its inhibitor – calpastatin – encoded by the *CAST* gene, play a key role in the beef tenderization process (Koohmaraie, 1996). To date several markers and significant associations have been shown both in *CAPN1* (e.g. Page et al., 2002; Casas et al., 2006) and *CAST* genes (e.g. Schenkel et al., 2006; Barendse et al., 2007) in different cattle populations; both genes have been localized in QTL regions for beef tenderness on BTA29 and BTA7, respectively (Smith et al., 2000; Casas et al., 2003).

The quality of the fat is determined by fatty acid composition which may also contribute directly to beef flavour, juiciness, and nutrition value, and indirectly to tenderness (Taniguchi et al., 2004; Matsuhashi et al., 2011). Stearoyl-CoA desaturase (SCD) is the enzyme responsible for conversion of saturated fatty acids into monounsaturated fatty acids (MUFA) in mammalian adipocytes. The *SCD* gene is localized on BTA26. The *SCD* genotype was associated with fatty acid profile in Japanese Black cattle (Taniguchi et al., 2004). Fatty acid synthase

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(FASN) is a multifunctional enzyme complex that catalyzes the synthesis of long-chain saturated fatty acids (SFA). It is hypothesized that variation in TE domain (exons 39–42, responsible for fatty acid synthesis termination) of the *FASN* gene would be a candidate for heritable differences in fatty acid composition (Zhang et al., 2008). Roy et al. (2001) mapped the bovine *FASN* gene to BTA19 in QTL for fatty acid composition of beef and milk. Many studies have reported the positive effect of *FASN* gene on fatty acid profile (e.g. Zhang et al., 2008; Oh et al., 2012).

In this study we investigated the association of markers *CAPN1* AF252504.2:c.947G>C and *CAST* NM174003.2:c.155C>T which have been reported by Barendse et al. (2007) as potential causative mutations for beef tenderness and marker *SCD* AB075020:c.878T>C which causes the substitution of the amino acid valine to alanine at position 293 of SCD1 protein and *FASN* AF285607:g.17924A>G which results in an amino acid replacement from threonine (ACC) to alanine (GCC) in the FASN TE domain. The objective of this study was to evaluate the genetic variability of the four previously reported polymorphisms (*CAPN1, CAST, SCD,* and *FASN*) and to assess the relationships between them and beef quality traits in crossbred cattle population (Czech Fleckvieh, Charolais, Beef Simmental, Galloway, Blonde d'Aquitaine).

MATERIAL AND METHODS

Animals and beef quality characteristics

A beef crossbred cattle population of 331 animals reared in less favoured agricultural areas (Czech Fleckvieh, Charolais, Beef Simmental, Galloway, Blonde d'Aquitaine) was fed with defined feeding type (pasture and hay or intensive feeding system based on corn silage) during the fattening period. According to the CSN 57 6510 (2003), the average age at slaughter was 650.4 days. Samples of *Musculus longissimus lumborum et thoracis (mllt)* were obtained from the carcass next day after slaughter. Carcass characteristics and cutting parts of carcass were measured according to Voříšková et al. (2011).

The intramuscular fat content (%) was determined by Soxhlet extraction, water binding capacity by a modification of Grau and Hamm's press method, tenderness by a Tira-test 27025 device, colour (mg/g) according to Hornsey (1956), pH_{48} by a pH-meter (WTW, Weilheim, Germany), colour characteristics (L^* – lightness, a^* – redness, b^* – yellowness) by a CM-2600d spectrophotometer (Konica Minolta Co. Ltd., Osaka, Japan), diameter of muscle fibres (μm) by a Leica DMR microscope with a DC100 camera and Leica DC Viewer software (Leica, Milan, Italy), and fatty acid profile (%) of muscle fat by gas chromatography according to Komprda et al. (2005).

Genotyping

Total genomic DNA was extracted by JETQUICK Blood and Cell DNA Spin Kit from blood stored with EDTA and by JETQUICK Tissue Spin Kit (both Genomed, Bad Oeynhausen, Germany) from *mllt* samples according to standard protocol and stored at –20°C.

The SNPs in *CAPN1 (*AF252504.2:c.947G>C), *SCD* (AB075020:c.878T>C), *CAST (*NM174003.2:c.155C>T), and *FASN* (AF285607:g.17924A>G) have been previously described (Page et al., 2002; Taniguchi et al., 2004; Barendse et al., 2007; Zhang et al., 2008, respectively). Multiplex PCR-RFLP assays were developed for simultaneous analysis to facilitate genotyping of *CAPN1* with *CAST* (multiplex 1) and *SCD* with *FASN* (multiplex 2). Primer pairs of *SCD, CAST,* and *FASN* were previously reported by Taniguchi et al. (2004), Barendse et al. (2007), and Zhang et al. (2008), respectively. The *CAPN1* primer pair (F: 5'-GAGCTGGCCCTCATAAGATAA-3' and R: 5'-TGAGAGTGAACCAGGATGC-3') was designed on the basis of bovine *CAPN1* gene sequence (AF252504) using Oligo 4.0 Primer Design Software (National Biosciences Inc., Plymouth, USA) – the specific amplified fragment was 343 bp long.

The multiplex PCRs were carried out on a Veriti® 96-Well Thermal Cycler (Applied Biosystems, Foster City, USA) in a volume of 15 μl containing 1× Combi PPP Master Mix (with 0.375 U *Taq* Purple polymerase) (Top-Bio, Prague, Czech Republic), 0.6μM of each primer of *CAPN1* and *CAST* or 0.4μM of each primer of *FASN* and 0.8μM of each primer of *SCD*, and 50–100 ng of bovine genomic DNA. The conditions for *CAPN1* and *CAST* multiplex PCR included an initial denaturation step of 7 min at 94°C followed by 30 cycles of 30 s at 94°C, 20 s at 64°C, and 20 s at 72°C, with a final extension at 72°C for 7 min. The conditions for

SCD and *FASN* multiplex PCR included an initial denaturation step of 7 min at 94°C followed by 38 cycles of 40 s at 94°C, 30 s at 62°C, and 50 s at 72°C, with a final extension at 72°C for 10 min. The successful amplifications (343 bp fragment of *CAPN1* with 101 bp fragment of *CAST* and 382 bp fragment of *FASN* with 209 bp of *SCD*) were verified by electrophoresis on 2% agarose gel containing TBE and ethidium bromide for visualization under ultraviolet light. The PCR products of multiplex 1 were digested with *Btg*I and *Msp*I (both New England BioLabs, Beverly, USA). Digestion was conducted at 37°C overnight and a 15-µl reaction solution included 5–10 µl of PCR products, 1.5× NEBuffer 4 (New England BioLabs), and 3 U of each restriction enzyme. The PCR products of multiplex 2 were digested with *Fau*I and *Msc*I (both New England BioLabs). Digestion was conducted at 37°C overnight and a 15-µl reaction solution included 5–10 µl of PCR products, 1.5× NEBuffer 4 (New England BioLabs), and 2 U of *Fau*I and 1 U of *Msc*I. The DNA fragments after digestion were separated by electrophoresis on 3–3.5% agarose gel. The variation at the position *CAPN1, CAST, SCD,* and *FASN* was resulting in the presence/absence of a *Btg*I and *Msp*I restriction site, or the presence/absence of *Fau*I and *Msc*I specific restriction site, respectively.

Statistical analyses

Data were collected in the years 2006–2010 and organized into two data sets. The first data set (188 animals) contained beef characteristics: tenderness – Warner-Bratzler shear force, colour characteristics – colour, L^*, a^*, b^*, pH, texture profile analysis, intramuscular fat content, water binding capacity, and cooking loss. The second data set contained 143 animals with information about fatty acid composition (SFA (saturated fatty acids), MUFA (monounsaturated fatty acids), PUFA (polyunsaturated fatty acids), 14:0 (myristic acid), 14:1 (myristoleic acid), 16:0 (palmitic acid), 16:1 (palmitoleic acid), 18:0 (stearic acid), and 18:1 (oleic acid)).

Genotypic frequencies were tested by χ^2-test. Statistical analyses concerning to beef were carried out in SPSS software (Version 16.0.1, 2007) for MS Windows using ANOVA Repeated Measures providing the Analysis of Variance for the same measurements that were made several times on each subject (*mllt*). The beef characteristics were measured during aging: on days 2, 16, 30, and 44 after slaughter for beef tenderness, and on days 2 and 44 after slaughter for fatty acid profile. The model included aging time as the within-subject factor and effect of sex, diet, sire, and genotype as between-subjects factors.

The equation was as follows:

$$Y_{ijklmnopq} = \mu + aging_i + sex_j + feed_k + sire_l + gene_m + age_n + muscle_o + t \times f_p + e_{ijklmnopq}$$

where:

$Y_{ijklmnopq}$ = phenotypic value of the analyzed trait
μ = population mean
$aging_i$ = aging period effect (2 or 4 intervals)
sex_j = fixed effect of sex
$feed_k$ = fixed effect of feeding type (pasture or corn silage)
$sire_l$ = fixed effect of sire
$gene_m$ = fixed effect of genotype
age_n = effect of age
$muscle_o$ = random effect of measured muscle
$t \times f_p$ = interaction of aging period and each fixed effect at all levels
$e_{ijklmnopq}$ = random error effect of each observation

The analyses were performed separately for each marker – *CAPN1* (*CC, CG* or *GG*), *CAST* (*CC, CT* or *TT*), *SCD* (*TT, TC* or *CC*), and *FASN* (*AA, AG* or *GG*). Results of the analysis between the *CAPN1* or *CAST* genotypes and beef tenderness and other beef quality characteristics are presented as the average measure over 4 measurements, in the case of *SCD* or *FASN* genotypes as the average measure over 2 measurements.

RESULTS AND DISCUSSION

CAPN1 and *CAST* genes

Barendse et al. (2007) considered the *CAPN1* c.947G>C is the marker useful in taurine animals and their results confirm the effect of this SNP on beef tenderness and together with polymorphism *CAST* c.155C>T show the significant epistasis. Moreover, there has been no simultaneous evaluation of *CAPN1* and *CAST* genes to assess their effect on meat tenderization in the Czech populations of commercial cattle.

The *CAPN1* marker (*CAPN1* c.947G>C) was polymorphic in our crossbred population, but

Table 1. Genotype and allele frequencies

Marker	Genotype frequencies		Allele frequencies	
CAPN1	*CC* (*n* = 2)	0.01	*C*	0.08
	CG (*n* = 27)	0.14		
	GG (*n* = 159)	0.85	*G*	0.92
CAST	*CC* (*n* = 12)	0.06	*C*	0.46
	CT (*n* = 147)	0.78		
	TT (*n* = 29)	0.16	*T*	0.54
SCD	*CC* (*n* = 40)	0.28	*C*	0.58
	CT (*n* = 85)	0.59		
	TT (*n* = 18)	0.13	*T*	0.42
FASN	*AA* (*n* = 18)	0.13	*A*	0.35
	AG (*n* = 64)	0.44		
	GG (*n* = 61)	0.43	*G*	0.65

genotype frequencies and allele distribution were not balanced (Table 1). Genotype frequencies for *CAPN1* revealed large differences between genotypes – a prevalence of the *GG* genotype (0.85) and a very low frequency (0.01) of *CC* genotype. The minor allele frequency (*C* allele) was 8%. The *CC* genotype was found only in 2 animals and was excluded from further association analyses.

The polymorphism *CAST* c.155C>T (marker *CAST*) was polymorphic in our crossbred population with relatively balanced allele frequencies (0.46 for *C* allele, 0.54 for *T* allele) (Table 1). The *CT* genotype was the most frequently represented genotype and only 12 animals were the carriers of the *CC* genotype.

The *CAPN1* marker has been previously reported and associated with meat tenderness. This marker showed significant association ($P < 0.05$) with shear force in commercial population (Simmental × Aberdeen Angus) when animals homozygous for the *C* allele had lower Warner-Bratzler shear force than animals with *CG* and *GG* genotypes (Page et al., 2004). The polymorphism of *CAPN1* in exon 9 produces relatively conservative glycine-to-alanine substitution at position 316 in the protein located in Domain II, which has been identified as the proteolysis domain. So the alternation in this domain could alter the activity of the protein and thus affect the meat tenderization (Page et al., 2002). In our association study the genotypes of *CAPN1* marker did not significantly influence any tenderness characteristics; the *CAPN1* marker was significantly associated only with pH with the trend *CG* > *GG* when the significant differences between genotypes were found – *CG* a *GG* with significance at the level of $P = 0.01$ (Table 2). There was an unclear pattern. Many studies (e.g. Page et al., 2004; Van Eenennaam et al., 2007) confirmed positive effect of *C* allele of *CAPN1* marker on meat tenderness in different cattle populations (*Bos taurus*), but in our commercial crossbred population the influence of favourable allele on beef tenderness was not demonstrated. Our results tended to confirm the breed-specific hypothesis of Allais et al. (2011) who showed the significant relationship of *G* allele to tougher meat just in the Charolais ($P = 0.01$) and Limousine ($P = 0.02$). On the other hand, the consistency of the results in *Bos taurus* and similar effect of *C* allele across a wide variety of populations (where the associations with beef tenderness were significant) suggest that *CAPN1* marker could be the useful marker. Although the significant effect of *CAPN1* marker on beef tenderness was not demonstrated in our commercial crossbred population, it can be assumed that this inconsistency was caused by unequal genotype distribution so the association study should include more individuals with the *CC* genotype.

For the *CAST* marker, a highly significant difference ($P = 0.0001$) between *CC* and *CT* genotypes and significant ($P = 0.024$) difference between *CC* and *TT* genotypes for Warner-Bratzler shear force were found (Table 2). Higher Warner-Bratzler shear force and so lower beef tenderness was observed for *CC* genotype. Calpastatin inhibits calpain 1 through the B domain, however, the SNP *CAST* c.155C>T causing amino acid change at position 52 of calpastatin is located at the L domain, which has not been assigned a role in the interaction between calpain and calpastatin molecules until recently. The amino terminal part of calpastatin molecule part, including the L domain, was known to be responsible for binding to biological membranes at acidic phospholipids and to catalytic DII domain of calpain and the calpain undergoes a conformational change, increasing its ability to act as a protease. The changes may occur either where calpastatin binds to cell membranes or where it binds calpain 1 altering the resulting phenotype for meat tenderness (Barendse et al., 2007).

Except the appointed association of *CAST* marker with meat tenderness, we found highly significant ($P = 0.001$) differences between *CT* and *TT* genotypes and pH – the animals with *CT* genotype had higher pH compared to animals with *TT*. In

Table 2. Association between *CAPN1* and *CAST* genotypes and beef tenderness and other quality traits in crossbred cattle

Trait	Genotype effects†			*P*-value
CAPN1	*CC* (*n* = 2)	*CG* (*n* = 27)	*GG* (*n* = 159)	
WB	–	90.592 ± 4.521	89.428 ± 2.905	
*a** (redness)	–	11.661 ± 0.305	11.570 ± 0.205	
*b** (yellowness)	–	9.693 ± 0.269	9.617 ± 0.181	
*L** (lightness)	–	37.428 ± 0.523	37.292 ± 0.353	
Colour	–	3.824 ± 0.139	3.916 ± 0.094	
pH	–	5.555 ± 0.015^{a}	5.547 ± 0.010^{a}	*
T	–	245.357 ± 14.708	250.957 ± 9.324	
IMF	–	1.664 ± 0.309	1.871 ± 0.309	
WBC	–	84.070 ± 0.916	82.666 ± 0.617	
CL	–	31.653 ± 0.548	31.428 ± 0.352	
CAST	*CC* (*n* = 12)	*CT* (*n* = 147)	*TT* (*n* = 29)	
WB	109.304 ± 6.352^{Ab}	88.053 ± 2.744^{A}	93.203 ± 4.612^{b}	***/*
*a** (redness)	10.950 ± 0.428	11.613 ± 0.201	11.695 ± 0.318	
*b** (yellowness)	9.223 ± 0.375	9.606 ± 0.176	9.917 ± 0.279	
*L** (lightness)	37.840 ± 0.734	37.231 ± 0.345	37.840 ± 0.547	
Colour	3.711 ± 0.196	3.931 ± 0.092	3.808 ± 0.146	
pH	5.546 ± 0.020	5.552 ± 0.009^{A}	5.509 ± 0.015^{A}	***
T	236.105 ± 23.249	248.391 ± 9.022	262.829 ± 15.179	
IMF	2.102 ± 0.435	1.823 ± 0.204	1.734 ± 0.324	
WBC	83.042 ± 1.299	82.886 ± 0.610	83.294 ± 0.967	
CL	31.236 ± 0.643	32.076 ± 0.852	32.831 ± 0.184	

WB = Warner-Bratzler shear force, T = tenderness, IMF = intramuscular fat content, WBC = water binding capacity, CL = cooking loss, *n* = number of animals with a relevant genotype

†Least Squares Means ± standard error of beef quality traits

*$P < 0.05$ (lowercase letters), **$P < 0.01$, ***$P < 0.001$ (uppercase letters)

animals with *CC* genotype the highest value of pH was not observed and, in addition, there were no significant differences between other genotype pairs. Our results confirm the results of Barendse et al. (2007) – the *CC* genotype is associated with the higher Warner-Bratzler shear force and tougher meat. In contrast to our results, Barendse et al. (2007) observed the intermediate values of shear force for *CT* genotype and the lower values for *TT* genotypes; our results (*CC* > *TT* > *CT*) suggest a possible effect of overdominance.

SCD and *FASN* genes

The SNP in c.878T>C in *SCD* gene was polymorphic and the frequencies of the minor allele (*T*) and the rare genotype (*TT*) were 0.42 and 0.13, respectively (Table 1). Our association test of the *T/C* polymorphism of *SCD* marker revealed a positive effect of allele *C* (alanine) on myristoleic acid (C14:1) content (Table 3) – we observed the significant difference between *CC* and *CT* genotypes ($P = 0.002$), *CT* and *TT* ($P = 0.026$), and *CC* and *TT* ($P = 0.0001$) with the trend *CC* > *CT* > *TT*. Similar results for the effect of *SCD* genotype on myristoleic acid and also on stearic acid were reported by Bartoň et al. (2010) in Czech Fleckvieh bulls. In our study the influence of *CC* genotype on lower stearic acid (C18:0) content was not confirmed – we observed the trend *CC* < *CT* < *TT* but above the significance level. Matsuhashi et al. (2011) published also highly significant effect of this SNP on the content of myristic, stearic, and

Table 3. Association between *SCD* and *FASN* genotypes and fatty acid composition in crossbred cattle

Trait	Genotype effects†			*P*-value
SCD	*CC* (*n* = 40)	*CT* (*n* = 85)	*TT* (*n* = 18)	
SFA	49.678 ± 0.718	50.364 ± 0.625	49.859 ± 0.971	
MUFA	45.249 ± 0.743	44.461 ± 0.647	45.230 ± 1.006	
PUFA	5.076 ± 0.329	5.159 ± 0.287	4.907 ± 0.446	
14:0	2.480 ± 0.116	2.549 ± 0.101	2.488 ± 0.157	
14:1	0.500 ± 0.046^{A1}	0.377 ± 0.040^{b1}	0.289 ± 0.062^{Ab}	***/**/*
16:0	27.825 ± 0.454	27.493 ± 0.395	26.978 ± 0.615	
16:1	2.920 ± 0.159	2.879 ± 0.138	2.996 ± 0.215	
18:0	19.142 ± 0.620	20.095 ± 0.540	20.175 ± 0.840	
18:1	41.630 ± 0.671	41.038 ± 0.584	41.763 ± 0.909	
FASN	*AA* (*n* = 18)	*AG* (*n* = 64)	*GG* (*n* = 61)	
SFA	49.183 ± 0.942	50.329 ± 0.689	50.096 ± 0.610	
MUFA	45.519 ± 0.980	44.543 ± 0.717	44.821 ± 0.635	
PUFA	5.321 ± 0.433	5.100 ± 0.317	5.078 ± 0.280	
14:0	2.678 ± 0.151	2.622 ± 0.110^{a}	2.461 ± 0.097^{a}	*
14:1	0.479 ± 0.062	0.441 ± 0.046^{a}	0.375 ± 0.040^{a}	*
16:0	27.919 ± 0.591	27.968 ± 0.433^{a}	27.285 ± 0.383^{a}	*
16:1	3.388 ± 0.200^{a1}	3.049 ± 0.146^{ab}	2.797 ± 0.129^{1b}	**/*
18:0	18.353 ± 0.808^{a}	19.519 ± 0.591	20.119 ± 0.524^{a}	*
18:1	41.494 ± 0.883	40.874 ± 0.646	41.470 ± 0.572	

†Least Squares Means ± standard error of beef quality traits

SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, *n* = number of animals with a relevant genotype

*P < 0.05 (lowercase letters), **P < 0.01 (numeral), ***P < 0.001 (uppercase letters)

oleic acids and also on MUFA percentage. Also Bartoň et al. (2010) published other significant associations – negative effect of allele *C* on PUFA percentage, positive effect of allele *C* on oleic acid or on MUFA percentage.

The allele and genotype frequencies of SNP g.17924A>G in *FASN* gene are presented in Table 1. Occurrence of the *A* allele and the *AA* genotype in the studied population was at the level of 0.35 and 0.13, respectively.

The significant influence of polymorphism *FASN* g.17924A>G on fatty acid profile was confirmed by the study of Zhang et al. (2008) where the *GG* genotype was associated with higher percentage of oleic acid or higher percentage of MUFA, and also by the study of Oh et al. (2012) where the positive effect of *G* allele (alanine) on MUFA percentage and negative effect on PUFA in Korean cattle were proved. The results of our study did not confirm the significant influence on SFA, MUFA or PUFA percentage, however we observed a significant difference between *AG* and *GG* genotypes (P = 0.035, *AG* > *GG*) and myristooleic acid content (Table 3). We also observed a significant positive effect of allele *A* on palmitoleic acid percentage (*AA* > *AG* > *GG)* and on stearic acid content (*AA* > *GG*). A notable difference was observed also between the *AG* and *GG* genotypes (P = 0.043, *AG* > *GG*) and myristic acid content, and palmitic acid (P = 0.043, *AG* > *GG*).

According to the obtained results we can confirm positive effect of the *A* allele (threonine) on a higher content of unsaturated fatty acids but the *A* allele was associated with a lower content compared to *G* allele. Matsuhashi et al. (2011) revealed independent influence of *SCD* and *FASN* polymorphisms on the fatty acid composition in the *longissimus thoracis* muscle in Japanese Black cattle. Their results also

suggest that the analyzed SNPs in *FASN* and *SCD* genes are appropriate markers for the improvement of fatty acid profile in fat.

CONCLUSION

The presented results allow to confirm previously reported associations of *FASN, SCD,* and *CAST* polymorphisms with beef tenderness and fatty acid profile. Thus in our crossbred population the polymorphism *FASN* g.17924A>G appeared to be a very useful marker for selection of animals according to the fatty acid profile while the polymorphism *SCD* c.878T>C or *CAST* c.155C>T for beef tenderness. On the other hand, the association analysis of marker *CAPN1* c.947G>C was strongly influenced by genotype frequencies misbalance and we cannot confirm the effect on beef tenderness in our crossbred population. As shown in Pannier et al. (2010), it must be highlighted that some associations found to date were based on a limited number of breeds and on small sample sizes and family studies, hence further investigations have often failed to validate initial observations. The use of these gene polymorphisms directly for marker-assisted selection should be estimated in a large number of animals and further studies are needed to confirm the associations and to define more exactly the molecular mechanisms in which these genes are involved.

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