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Genetic polymorphisms at candidate genes affecting fat content and fatty acid composition in Modicana cows: effects on milk production traits in different feeding systems

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Feeding greatly affects milk yield and composition. The research is highlighting the potential of genetic polymorphism at some loci to affect milk yield and quality traits. These loci can be up/down regulated depending on the production environment; therefore, we hypothesized that milk yield and composition could differ when cows with different genotype at SCD, DGAT1 *and* ABCG2 *loci are reared in different feeding systems. The polymorphisms of* SCD, DGAT1 *and* ABCG2 *genes were investigated in Modicana breed. In all, three polymorphic sites, responsible for the genetic variation of quantitative trait loci and therefore defined quantitative trait nucleotides, were genotyped: the transition g.10329C > T in 5th exon determines a substitution p.A293V in the* SCD, *the dinucleotide mutation g.10433-10434AA > GC in 8th exon responsible for p.K232A substitution in the* DGAT1 *and the transition g.62569A > C in the 14th exon responsible for p.Y581S substitution in the* ABCG2 *gene. In the sample of 165 Modicana cows,* SCD *and* DGAT1 *genes resulted polymorphic; the alleles g.10329T and g.10433-10434GC were the most frequent in* SCD *and* DGAT1 *(0.73 and 0.91) respectively, whereas* ABCG2 *locus was monomorphic for allele A (p.581Y). Sequencing analysis was carried out on 14 samples with different genotypes to confirm the results of the PCR-RFLP protocols. Based on the genotypes at* SCD *locus, 47 Modicana cows were selected for the nutritional trial: 24 cows in a semi-intensive farm, with 2 h/day grazing on natural pasture, and 23 cows in an extensive farm, with 8 h/day grazing on natural pasture. Monthly, milk yield and composition were evaluated and individual milk samples were analyzed for fatty acids composition by gas chromatography. No differences in milk yield, fat, protein, lactose, casein and urea were associated to* SCD *genotype. Feeding systems affected milk yield and composition. No significant genotype × feeding system interaction was observed for milk yield and composition. Fatty acids composition was significantly affected only by the feeding system. Significant interactions were found between* SCD *genotype and feeding system for six fatty acids: 4:0, 6:0, 8:0, 10:0, 12:0 and* t*11 18:1. We concluded that the feeding system was the factor that mostly affected milk production and composition; moreover, our results do not confirm what reported in literature as regard the effect of the* SCD *polymorphism on milk fatty acid composition. The high amount of pasture seemed to have resized the* SCD *polymorphism effects because of the different fatty acids composition of the diet.*

Keywords: *ABCG2*, *DGAT1*, *SCD*, feeding, milk quality

Implications

This study provides new information on the polymorphism of three quantitative trait loci (QTL) in Modicana dairy cow. The investigated QTL are usually associated with variation in milk fat content and fatty acid (FA) composition; however, this study does not confirm the findings obtained with other breeds. Our results suggest that the effect of the genetic polymorphism on these milk traits may depend by the genetic background and the feeding system. In particular, the high amount of pasture seemed to resize the stearoyl-CoA desaturase (*SCD*) polymorphism effects because of the different FAs composition of the diet.

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Introduction

Modicana is an endangered Italian cattle breed, traditionally reared in Sicily for milk, that is mainly addressed to the production of typical 'pasta filata' Ragusano cheese labelled with a Protected Designation of Origin. As other ancient local breeds, Modicana deserves to be preserved as cultural

heritage but also for its economic, social and environmental role. European Union policy supports the conservation of local populations taking into account that, as compared to cosmopolitan breeds, their products are often characterized by a higher sensorial, nutritive and technological properties arising from the feeding system. Traditionally, Modicana is reared according to an extensive (EX) system, which is essentially based on pasture with no, or limited supplementation of concentrate during the grazing season; however, semi-intensive (SI) farming is also used. Similarly to the EX, the SI feeding system is based on forage, but the time spent on pasture is lower and level of concentrate supplementation is higher than the EX system.

It has been widely demonstrated that milk quality traits, including fat content and FAs composition, can be strongly affected by feeding (Chilliard *et al*., 2007). In particular, pasture-based diets are known to confer characteristic aroma, colour and biologically active and healthy molecules to dairy products. Even if feeding remains the main tool to manipulate milk FA profile, an increasing number of evidences is highlighting the potential of genetic polymorphism at some loci to affect milk yield and composition. Among these, acyl-CoA:diacylglycerol acyltransferase 1 (*DGAT1*) g.10433-10434AA > GC (Grisart *et al*., 2002), ATP binding cassette, subfamily G, member 2 (*ABCG2*) g.62569A > C (Cohen-Zinder *et al*., 2005) and *SCD* g.10329C > T (Macciotta *et al*., 2008) have been widely studied in different breeds but not in Modicana. Acyl-CoA:diacylglycerol acyltransferase 1 is a key enzyme in triglycerides synthesis (Schennink et al., 2008), *ABCG2* seems to be involved in the transport of the cholesterol into milk, whereas *SCD* catalyzes the addition of a double bond in Δ^9 position in FAs. These polymorphisms, responsible for the observed variation of QTL, can be defined as quantitative trait nucleotides (QTN). In addition, it has been observed that genes of QTL can be up/down regulated depending on the production environment (Lillehammer *et al*., 2009).

Modicana cattle breed has been scarcely investigated for its genetic polymorphism, except for milk protein genes (Ceriotti *et al*., 2004) and melanocortin 1 receptor which is one of the main genes implicated in the determination of the coat colour in mammals (Guastella *et al*., 2011), whereas no information is available on the polymorphism of *DGAT1*, *SCD* and *ABCG2* for this breed.

In the light of above, the aim of this research was to describe, for the first time in Modicana, the polymorphism of three QTN (*DGAT1*, *SCD* and *ABCG2*) involved in lipid metabolism and to evaluate the effect of the polymorphism on milk quality traits and FA composition in two traditional feeding systems characterized by a different use of pasture (8 *v.* 2 h/day). We hypothesized that milk yield and quality could differ among cows carrying different alleles at these loci when reared in different feeding systems. Therefore, the interaction between genetic polymorphism and feeding systems was investigated.

Material and methods

The experiment was conducted from October 2014 to June 2015 in two Modicana dairy cow farms located in the province of Ragusa (Southern of Italy) and characterized by two different feeding systems (respectively, SI and EX). In the SI farm (36°56′47″ N and 14°41′50″ E; 639 m above sea level), the diet consisted of stall feeding (hay and concentrate) and 2 h of daily grazing. In the EX farm (36°52′53″ N and 14° 33′51″ E; 308 m above sea level), feeding consisted primarily on pasture (8 h/day). *Malva neglecta*, *Chrysanthemum coronarium*, *Calendula arvensis* and *Carduus spp.* dominated the botanical composition of pasture. A total of 165 individual blood samples were collected in 10 ml vacutainer tubes (K3-EDTA), specifically 96 from SI and 69 from EX. Sampling was carried out to avoid animals closely related to each other, according to the information obtained by farmers and genealogical data.

Genetic characterization

Genomic DNA was extracted from white blood cells using the EUROGOLD DNA Blood Mini Kit, following the protocol provided by the manufacturer (EuroClone S.p.A., Pero (MI), Italy). The concentration was measured by using the NanoDrop 1000 Spectrotometer, and brought to 30 to 50 ng/μl. Genotypes at *ABCG2*, *DGAT1* and *SCD* loci have been determined using different PCR-RFLP and aCRS PCR-RFLP methods. Each investigated polymorphism causes an amino acid substitution in the protein sequence (Supplementary Table S1). Amplifications were performed in a 30-μl reaction volume for 35 cycles using a GenAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA) thermal cycler according to Komisarek and Dorynek (2009) and Komisarek *et al*. (2011). Primers pairs, temperature of annealing, size of the amplicons (bp), restriction endonuclease and restriction patterns are reported in the Supplementary Table S2. In order to confirm the results of the PCR-RFLP methods, fragments of 292, 378 and 333 bp were sequenced in a subsample of 14 cows with different *ABCG2*, *DGAT1* and *SCD* genotypes. Polymerase chain reactions were performed by using the same primer pairs and conditions as the PCR-RFLP protocols. Amplicons were purified using the Wizard® SV Gel and PCR Clean-Up System (Promega Corporation, Madison, WI, USA) and then eluted in 20 μl of pure water. The PCR products obtained by using ddNTPs triphosphate labelled with fluorophores (BigDye Terminator, Applied Biosystems) were purified in the CENTRES-SEP COLUMNS (Princeton Separation, Inc.) and then sequenced on an ABI PRISM 3130 Genetic Analyser equipped with Sequencing Analysis software (Applied Biosystems). The output sequences were compared with the respective reference sequences (GenBank Acc. Num. AJ871176, AY065621 and AY241932 to ABCG2, DGAT1 and SCD1, respectively) by the alignment with the software CLUSTALX 1.8 (Thompson *et al*., 1994). Departure from Hardy–Weinberg genetic equilibrium was evaluated by χ^2 test.

Animal and experimental design of the feeding trial

According to the observed polymorphism, which is detailed later in the 'Results' section, *SCD* was the only locus that allowed the creation of groups with a balanced presence of genotypes (TT, TC and CC) in the two farms. Therefore, three experimental groups were formed only on the basis of the genotypes at *SCD* locus. A total of 47 cows, 24 from SI and 23 from EX, at their third or fourth lactation, homogeneous for days of lactation (75.3 ± 20.1 and 88.7 ± 31.3 days for SI and EX, respectively) and milk yield (10.1 ± 1.3 and 9.0 ± 1.8 kg/day for SI and EX, respectively) were selected. Within each farm, the *SCD* genotypes were distributed as follows:

- SI: TT, 13 cows; TC, 4 cows; CC, 7 cows; and
- EX: TT, 9 cows; TC, 8 cows; CC, 6 cows.

All the selected individuals had the AA genotype for the *ABCG2* locus and the GC/GC (p. 232AA) genotype for the *DGAT1* locus. Feeding management in the two farms was performed as follows: SI cows were fed 5 kg of vetch and oats hay, 5 kg of a commercial concentrate (chemical composition is reported in the Supplementary Table S3) and grazed 2 h/day on natural pasture; EX cows were fed 5 kg of vetch and oats hay and grazed 8 h/day on natural pasture.

Data collection and analytical determinations

Monthly, individual milk yield was recorded and individual milk samples, consisting of proportional volumes from the morning and evening milk, were collected. Fat, protein and lactose were determined using an automated Fourier transform IR absorption spectrophotometric analyser (Combi-foss 6000; Foss Electric, Hillerød, Denmark). Fat was extracted from 50 ml of individual milk according to Luna *et al.* (2005) and 25 mg of lipid was converted to fatty acid methyl esters (FAME) by base-catalyzed transesterification (Christie, 1982), using 0.5 ml of sodium methoxide in methanol 0.5 N and 1 ml of hexane. Nonadecanoic acid was used as an internal standard at concentration of 1 mg/ml. Fatty acid methyl esters were analyzed in duplicate on a Trace Thermo Finnigan GC equipped with a flame ionization detector and a 100 m × 0.25 mm i.d. fused-silica capillary column (SP-2560; Supelco, Inc., Bellefonte, PA, USA). Helium was the carrier gas at a constant flow of 1 ml/min. Total FAME profile in a 1 μl sample volume at a split ratio of 1 : 80 was determined using the following GC conditions: the oven temperature was programmed at 50°C and held for 4 min, then increased to 120°C at 10°C/min, held for 1 min, then increased up to 180°C at 5°C/min, held for 18 min, then increased up to 200°C at 2°C/min, held for 15 min and then increased up to 230°C at 2°C/min, held for 19 min. The injector and detector temperatures were at 270°C and 300°C, respectively. Fatty acid methyl esters identification was based on the retention time comparison with commercially available standard mixture of FAME (Nu-Chek Prep Inc., Elysian, MN, USA; Larodan Fine Chemicals, Malmo, Sweden). Response factors to FID were calculated for individual FA with respect to the internal standard and intra-assay CV for each FA were calculated by using a reference standard butter (CRM 164; Community Bureau of Reference, Brussels, Belgium) and individual milk sample (Supplementary Table S4), detection threshold of FA was 0.001 mg/g FA. Fatty acids were expressed as g/100 g of total FAs.

Statistical analysis

Individual data for milk yield and composition (fat, protein, lactose, FA profile) were analyzed using the GLM procedure for repeated measures of SPSS (SPSS for Windows; SPSS Inc., Chicago, IL, USA). The analysis included the main effect of *SCD* genotype, feeding system, period and the interaction genotype × feeding system. The individual cow was included as a random factor. The following model was adopted:

$$Y_{ijkl} = \mu + F_i + G_j + I_k(F) + (F \times G)_{ij} + e_{ijkl}$$

where Y_{ijkl} is the observation; μ is the overall mean; F_i the fixed effect of feeding system (i = EX; SI); G_j the fixed effect of SCD genotype (j = TT; TC; CC); I_k(F) is the random effect of the individual cow nested with the feeding system; $(F \times G)_{ij}$ is the interaction between feeding system and SCD genotype; e_{ijkl} is the residual error.

Pre-experimental data of milk yield were used as covariates for milk production and gross composition. When the covariance was not significant ($P > 0.05$), it was removed from the statistical model. Differences between means were tested by LSD. The individual cow was considered as the experimental units and significance was declared when $P \leqslant 0.05$.

Results

In the whole sample of 165 Modicana cows, three *DGAT1* (Table 1) and three *SCD* (Table 2) genotypes were found, whereas *ABCG2* locus was found to be monomorphic for the

Table 1 *Genotype, genotype frequencies and allele frequencies at the DGAT1 locus in 165 Modicana cows*

	Genotype (*n. of animal*)			Genotype frequencies			Allele frequencies	
	GC/GC	GC/AA	AA/AA	GC/GC	GC/AA	AA/AA	GC	AA
EX farm	55	14	0	0.80	0.20	0.00	0.90	0.10
SI farm	82	11	3	0.85	0.12	0.03	0.91	0.09
Total	137	25	3	0.83	0.15	0.02	0.91	0.09

EX = extensive; SI = semi-intensive.

allele A (p.582Y). The genotypes g.10433-10434GC > GC and g.10329TT were the most frequent in *DGAT1* and *SCD* (0.83 and 0.63, respectively). Furthermore, the distribution of the genotypes in the two farms was very uneven; for instance, g.10433-10434AA > AA genotype was not detected in the EX farm. Deviation from Hardy–Weinberg equilibrium was observed only in *SCD* locus ($\chi^2 = 35.19$; $P \leqslant 0.001$). The sequence analysis conducted on a representative sample of the different genotypes confirmed the results obtained by PCR-RFLP and aCRS PCR-RFLP methods and showed no new mutation in the analyzed exonic regions of 292, 378 and 333 bp of *ABCG2*, *DGAT1* and *SCD* genes, respectively.

Table 3 reports the main effects of *SCD* genotype and feeding system on milk yield and gross composition. No significant differences in milk yield, fat, protein, lactose, casein and urea were associated to *SCD* genotype. Feeding system affected milk yield, significantly higher in SI farm, and lactose, significantly higher in EX farm. No significant genotype × feeding system interaction was observed for milk yield and composition.

Table 4 reports the main effects of *SCD* genotype and feeding system on FAs profile. On average, the polymorphism at *SCD* locus did not affect FA composition. Only the percentage of 11:0 was higher when the g.10329C allele was present at homozygous or heterozygous status. On the contrary, the feeding system affected almost all the FAs. Specifically, short chain FAs (4:0÷8:0), 18:0, *t*11 18:1, *c*9-*c*12-*c*15 18:3 (ALA), *c*9-*t*11 18:2 (RA) were higher, whereas medium chain FAs (12:0÷16:0), most of the odd and branched chain FAs (OBCFAs), and sum of monounsaturated FAs (MUFA) were lower in EX *v.* SI. A significant interaction between *SCD* genotype and feeding system was found for 4:0, 6:0, 8:0, 10:0, 12:0 and *t*11 18:1 (Figure 1). In particular, within the EX system, the three genotypes showed significant different values for 4:0÷12:0 and *t*11 18, decreasing from g.10329CC to g.10329TT with intermediate values in g.10329TC cows. The opposite was found in the SI farm, where g.10329TT genotype showed significantly higher percentage ($P \leqslant 0.05$) for 4:0, 6:0, 8:0, 10:0 and *t*11 18:1 as compared to the g.10329CC group. Proportion of 4:0 and 6:0 were significantly higher in TC than CC cows; moreover, 8:0 did not show significant differences between the homozygous groups, whereas 10:0 and *t*11 18:1 were lower in TC in comparison with TT. The percentage of 12:0 was not significantly affected by genotypes in cows reared in the SI system.

Table 2 *GC/GC, GC/AA and AA/AA genotype corresponded to p.232AA, p.232AK and p.232KK phenotype, respectively. Genotype, genotype frequencies and allele frequencies at the SCD locus in 165 Modicana cows*

	Genotype (*n. of animal*)			Genotype frequencies			Allele frequencies	
	TT	TC	CC	TT	TC	CC	T	C
EX farm	22	31	16	0.32	0.45	0.23	0.54	0.46
SI farm	81	4	11	0.84	0.04	0.12	0.86	0.14
Total	103	35	27	0.63	0.21	0.16	0.73	0.27

EX = extensive; SI = semi-intensive.
TT, TC and CC genotype corresponded to p.293VV, p.293AV and p.293AA phenotype, respectively.

Discussion

The sample of 165 Modicana cows was monomorphic for the wild allele *ABCG2*A. Similar results are observed in many breeds all over the world when the mutation that determines the variant p.581S has not been found, as in the case of *Bos indicus* breeds, or when the phenotype p.581YY is largely predominant (Komisarek and Dorynek, 2009; Sharma *et al.*, 2016), with the rare exceptions of two Turkey indigenous cattle breeds (Ates *et al.*, 2014).

At the *DGAT1* locus the GC allele (g.10433-10434GC), coding for Alanine in position 232, is the most frequent in this sample of Modicana (91%) and the homozygous phenotype (p.232 AA) is largely predominant (82%) with quite similar frequencies in the two farms. This finding is

Table 3 *Effects of SCD genotype and feeding system on milk yield and quality traits in Modicana cow*

	Genotype (G)			Feeding System (F)		Significance			
	TT	TC	CC	EX	SI	G	F	G × F	SEM
Milk yield (g/day)	9.2	9.8	9.7	8.6[a]	10.5[b]	0.789	0.007	0.896	0.39
Fat (%)	3.9	4.2	4.0	4.0	4.1	0.335	0.637	0.983	0.07
Protein (%)	3.6	3.8	3.6	3.6	3.7	0.120	0.255	0.059	0.03
Lactose (%)	4.5	4.4	4.4	4.6[a]	4.3[b]	0.791	<0.001	0.719	0.03
Casein (%)	2.7	2.9	2.8	2.7	2.9	0.356	0.095	0.665	0.03
Urea (mg/dl)	22.4	24.6	24.5	24.7	22.9	0.744	0.512	0.460	1.33
Fat (g/day)	367	388	378	343	414	0.920	0.022	0.744	13.8
Protein (g/day)	337	358	343	313	378	0.968	0.021	0.676	13.3
Lactose (g/day)	419	128	420	405	439	0.827	0.637	0.502	18.6
Casein (g/day)	256	273	263	238	290	0.912	0.071	0.565	10.5

EX = extensive system; SI = semi-intensive system.
TT, TC and CC genotype corresponded to p.293VV, p.293AV, p.293AA phenotype, respectively.
[a,b]Values within a row with different superscripts differ significantly at $P \leqslant 0.05$.

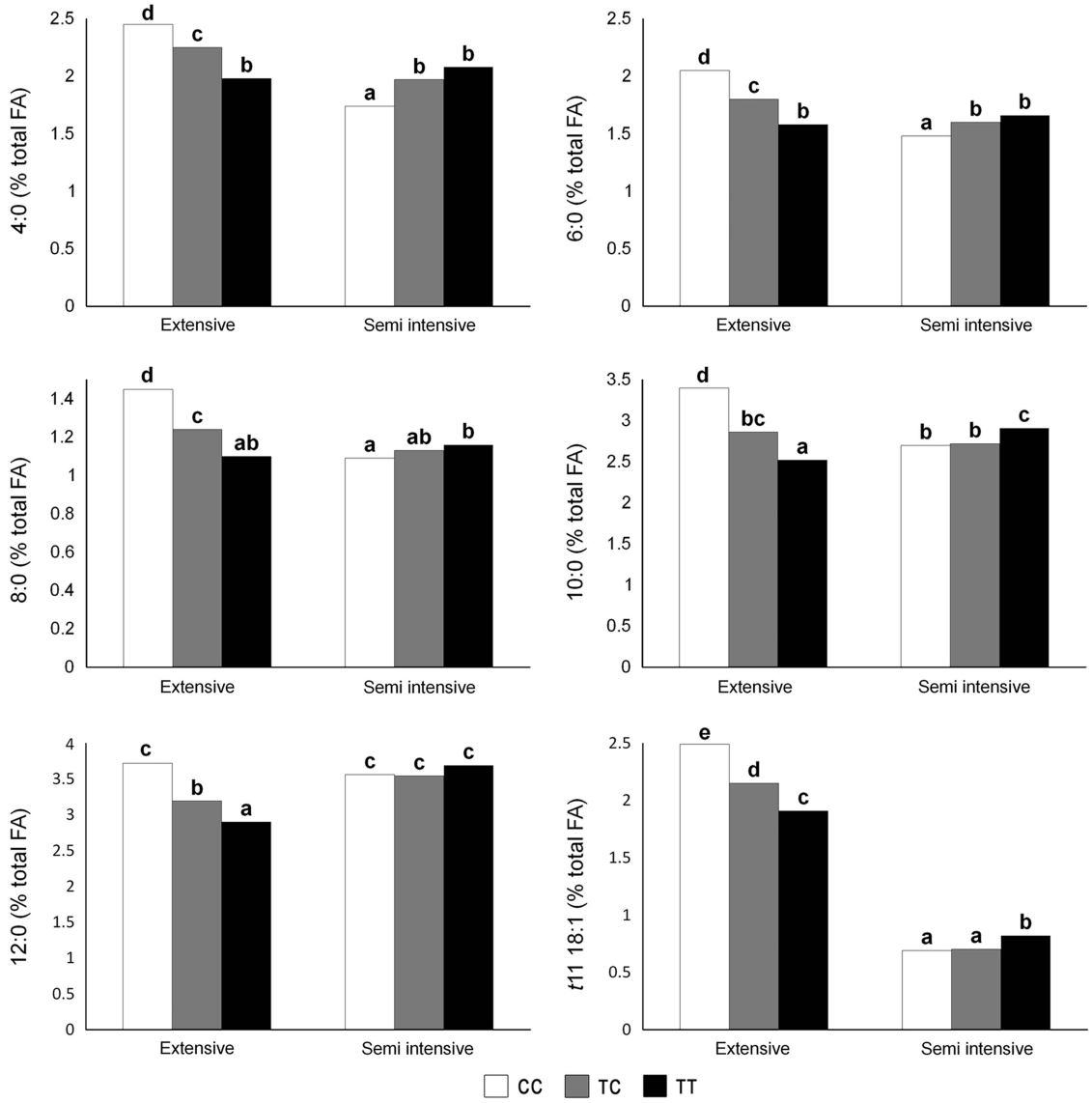

Figure 1 Interacting effect of the feeding system (extensive and semi intensive) and of the genotype at *SCD* locus (CC, TC and TT) on milk fatty acids (FA) in Modicana cow milk.
[a-e]Different superscript letters indicate differences ($P \leqslant 0.05$) between values tested by LSD.

consistent with several studies reporting p.232A as the predominant variant in a wide range of *Bos taurus* breeds (Kaupe *et al*., 2004; Näslund *et al*., 2008; Scotti *et al*., 2010 for a review). Only in Holstein cattle, bred worldwide, a more balanced distribution between the two alleles has been described, even if a wide range of variability across countries is also evident (Scotti *et al*., 2010). The genotype associated to the Lysine amino acid (p.232 KK), extremely rare in the Modicana, was found at low frequencies in the SI, whereas it was absent in the EX farm. As regards the low frequency of this variant, Gautier *et al*. (2007) and Conte *et al*. (2010) suggested that the frequency of the p.232K lately increased, particularly in Holstein breed, due to the intensive selection in favour of fat content.

A more balanced distribution of the genetic variability was observed at *SCD* gene. After the first study of Taniguchi *et al*. (2004) on Japanese Black cattle, the genetic polymorphism at *SCD* locus has been investigated in many dairy, beef and dual-purpose cattle breeds in different countries. At this locus, the comparison with the literature is quite difficult because of the different nomenclature used by the authors to describe the genetic variability at different level. In particular, the here investigated polymorphism can be defined as g.10329 C > T (according to the genomic sequence), c.C878T (taking into account the coding sequence) and p.A293V (at the protein level). Allele frequencies showed a wide range of variation in the breeds. Variant p.293A (allele g.10329C) was found to be largely predominant in Jersey (Moioli *et al*., 2007), in Italian Brown (Milanesi *et al*., 2008) and in local cattle breeds from South America (Carvajal *et al*., 2016). It was more frequent also in Valdostana (Moioli *et al*., 2007) and in Italian Holsteins (Macciotta *et al*., 2008) as in Japanese Black cattle (Taniguchi *et al*., 2004) and in Montbeliard farmed in Chile (Carvajal *et al*., 2016) but with more

Table 4 *Effects of SCD genotype and feeding system on milk fatty acid composition (g/100 g total fatty acids) in Modicana cow*

	Genotype (G)			Feeding System (F)		Significance			
	TT	TC	CC	EX	SI	G	F	G × F	SEM
4:0	2.1	2.1	2.0	2.2[a]	1.9[b]	0.766	0.006	0.005	0.05
6:0	1.8	1.7	1.6	1.8[a]	1.6[b]	0.253	0.006	0.003	0.04
8:0	1.3	1.2	1.1	1.3[a]	1.1[b]	0.110	0.020	0.007	0.03
10:0	3.0	2.8	2.7	2.9	2.8	0.127	0.304	0.007	0.07
11:0	0.35[a]	0.32[b]	0.29[c]	0.29[a]	0.36[b]	0.021	0.001	0.662	0.01
12:0	3.6	3.4	3.3	3.3[a]	3.6[b]	0.176	0.049	0.040	0.08
12:1	0.22	0.21	0.19	0.16[a]	0.25[b]	0.111	<0.001	0.363	0.01
14:0	11.6	11.1	11.1	10.6[a]	11.9[b]	0.549	0.002	0.480	0.21
*iso*15:0	0.41	0.42	0.40	0.35[a]	0.46[b]	0.542	<0.001	0.185	0.01
*anteiso*15:0	0.80	0.81	0.79	0.76[a]	0.83[b]	0.772	0.006	0.364	0.01
*c*9 14:1	1.03	0.91	0.82	0.69[a]	1.15[b]	0.067	<0.001	0.341	0.05
15:0	1.60	1.62	1.56	1.56[a]	1.63[b]	0.312	0.049	0.244	0.02
16:0	29.4	29.4	28.7	26.8[a]	31.5[b]	0.768	<0.001	0.970	0.53
*iso*17:0	0.45	0.48	0.47	0.44[a]	0.49[b]	0.487	0.002	0.209	0.01
*anteiso*17:0	0.58	0.60	0.56	0.46[a]	0.70[b]	0.390	<0.001	0.474	0.02
*c*9 16:1	1.3	1.5	1.4	1.2[a]	1.6[b]	0.396	<0.001	0.200	0.05
17:0	0.71	0.76	0.77	0.79[a]	0.70[b]	0.289	0.009	0.253	0.02
18:0	9.1	9.1	9.2	10.1[a]	8.2[b]	0.943	<0.001	0.797	0.23
t11 18:1	1.6	1.4	1.4	2.2[a]	0.7[b]	0.111	<0.001	0.006	0.11
*c*9 18:1	19.2	20.6	21.0	20.2	20.3	0.113	0.878	0.156	0.36
*c*9*c*12 18:2	1.6	1.6	1.7	1.6	1.6	0.077	0.865	0.369	0.03
20:0	0.21	0.21	0.22	0.20	0.23	0.813	0.093	0.263	0.01
*c*9*c*12*c*15 18:3	0.79	0.79	0.84	1.14[a]	0.47[b]	0.512	<0.001	0.560	0.05
*c*9*t*11 18:2	0.68	0.67	0.67	0.94[a]	0.41[b]	0.986	<0.001	0.637	0.04
20:4	0.23	0.14	0.18	0.21	0.16	0.658	0.511	0.634	0.03
ΣSFA	62.1	61.0	59.9	59.20	62.83	0.333	0.003	0.198	0.62
ΣMUFA	23.4	24.6	24.8	24.45	24	0.181	0.381	0.106	0.37
ΣPUFA	3.3	3.2	3.4	3.89	2.64	0.455	<0.001	0.979	0.12
Desaturation indexes*									
14:1 ratio*	0.08	0.07	0.07	0.06	0.09	0.112	<0.001	0.186	0.01
16:1 ratio*	0.04	0.05	0.05	0.04	0.07	0.102	0.002	0.065	0.01
18:1 ratio*	0.68	0.69	0.69	0.67	0.71	0.319	<0.001	0.083	0.05
*c*9*t*11 18:2 ratio*	0.32	0.34	0.33	0.30	0.36	0.547	<0.001	0.010	0.06

EX = extensive system; SI = semi-intensive system; SFA: saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.
TT, TC and CC genotype corresponded to p.293VV, p.293AV and p.293AA phenotype, respectively.
*Desaturation index calculated as reported by Kelsey *et al.*, 2003 is the ratio between *c*9- fatty acid and the sum of its saturated homologous plus the *c*9 fatty acids (e.g. *c*9 14:1/(14:0 + *c*9 14:1)).
[a,b]Values within a row with different superscripts differ significantly at $P \leqslant 0.05$.

balanced frequencies. In Modicana, g.10329T, coding for Valine in position 293, was found to be the predominant allele as in Podolica and in Red Pied analyzed by Milanesi *et al.* (2008) and in Piedmontese (Moioli *et al.*, 2007). The observed frequencies of *SCD* may reflect the different selection objectives followed in different breeds and countries to improve the milk composition (Gautier *et al.*, 2007). Finally, it is noteworthy that the result of the available studies are often conflicting, leading to the different identification of the ancestral allele which is g.10329C (p.293 A) for many authors, g.10329T (p.293V) for Taniguchi *et al.* (2004). In addition, the substitution effect of the alleles is not consistent in the different studies; it could be supposed that this is related to the presence of other genes located in BTA 26, such as *ELOVL3* (ELOVL FA elongase 3), *LIPA (lipase A, lysosomal acid type)* and *PLCE1* (phospholipase C epsilon 1) that are differently involved in the metabolism of lipids (Ogorevc *et al.*, 2009).

The principal aim of this study was to investigate the effect of genotype, at three *ABCG2*, *DGAT1* and *SCD* loci, the effect of feeding system and their interaction on milk yield and composition in Modicana cow. According to the literature, *ABCG2* seems to be involved in the transport of the cholesterol into milk (Cohen-Zinder *et al.*, 2005). The polymorphism p.Y581S was associated to variation of milk yield, fat and protein percentages in different breeds (Cohen-Zinder *et al.*, 2005; Mousavizadeh *et al.*, 2013). The ancestral allele A (p.581Y), the most frequent in all the breeds, was significantly associated with decreased milk yield and increased milk fat and protein percentage (Cohen-Zinder *et al.*, 2005;

Lillehammer *et al*., 2009). Regarding *DGAT1*, the polymorphism p.K232A is associated with an evident effect on milk FA composition. Schennink et al. (2008) found significant effects on 14:0, 16:0 and long chain FA desaturation indexes. In addition, Juhlin *et al.* (2012) found a significant *DGAT1* genotype effect on 16:0 and RA. Duchemin *et al*. (2013) reported *DGAT1* polymorphism was associated to most of the FAs and unsaturation indexes.

Unfortunately, the absence of variability observed for *ABCG2* gene in the considered population (165 cows) and the unbalanced distribution of the three genotypes found at *DGAT1* locus did not allow the formation of feeding groups with similar numerosity in the two farms. Consequently, the feeding study was conducted with animals that differed only for *SCD* genotype. Stearoyl-CoA desaturase enzyme catalyzes the addition of a double bond in Δ^9 position in FAs. Its activity can be estimated by using desaturation indexes calculated by expressing each product as a proportion of the precursor plus the product itself (Feng *et al*., 2007). As Schennink et al. (2008) reported that *DGAT1* and *SCD* polymorphism do not explain the same part of genetic variability on desaturation index, we decided to include in our feeding trial only cows characterized by the GC/GC genotype at the *DGAT1* locus. In principle, this choice allowed us to minimize the confounding effects that could have interfered in evaluating the role of the *SCD* on milk traits and FA composition.

In our experimental conditions FAs none of the unsaturation indexes, calculated as reported by Kelsey *et al*. (2003), were significantly affected by the *SCD* genotype. Desaturation of 14:0 is considered the most important index, due to the fact that the presence of *c*9 14:1 in milk only arises from the desaturation activity in the mammary gland, whereas products and precursors of the other FA pairs can originate from the diet or from the mobilization of body fat (Garnsworthy *et al*., 2010). In addition, desaturation index of 14:0 has the highest heritability (0.38) compared with the others (Garnsworthy *et al*., 2010). Although according to the literature 14:0 desaturation index is the most influenced by this locus, contrasting results are reported. A positive correlation between the C allele, coding for Alanine, in *SCD* and this index has been reported by Schennink et al. (2008), and Duchemin *et al*. (2013) on Holsein cattle and by Kgwatalala *et al*. (2009) in Jersey cattle. On the contrary, Conte *et al*. (2010) found higher 14:0-desaturation index in the TT genotype, coding for Valine, in Brown cattle. Regarding the relationship between the genotype at the *SCD* locus and the desaturation index of other FA, Schennink et al. (2008) report a lower index for 10:0 and 12:0 and higher index for 16:0 and RA in the cows homozygous for the p.293V phenotype. The same trend was found by Duchemin *et al*. (2013).

Conflicting findings reported in literature could be explained by the fact that the effect of polymorphism can differ across different populations, breeds because of specific genetic backgrounds and diet. Relatively small samples, with relatively large sampling errors, different analytical methods and statistical approaches, as well as the presence of other polymorphisms able to affect the investigate trait, can be cause of contradictory outcomes (Marchitelli *et al*., 2013).

Recently, Cecchinato et al. (2012) found the *SCD* g.10329T allele (p.293V) relevantly associated with an increase in fat percentage in cow milk. However, the same group carried out a wide association study to evaluate the effect of the polymorphism of 37 candidate genes on the detailed FAs profile in Brown Swiss bovine milk but *SCD* did not result a relevant gene in terms of association with FAs composition. The only exception was represented by 22:0 and *c*9 20:1 that were higher in milk fat of cows carrying g.10329T allele (Pegolo *et al*., 2016).

As expected, on average animals reared in the SI system showed a higher milk yield and protein content as compared to EX. Lactose resulted higher in animals reared in the extensive system. It is known that, among milk constituent, lactose fluctuations are minimal and mostly depend on the healthy status of the udder. Generally, mastitis has been negatively related with the concentration of lactose in milk; however, in the present trial no evidence of clinical mastitis was observed. Rather, it could be speculated that the higher percentage of lactose found in the EX milk in comparison with SI could depend on the different intake of minerals between the two feeding systems. In particular, McDowell (1996) reviewed that pasture often does not satisfy the mineral requirements of grazing cattle, which are inversely related with milk lactose. Surprisingly, fat was not affected by the feeding system. In fact, the low use of concentrate in the extensive than in the SI system should have resulted in a fat increase, due to both the higher fibre content in the diet and the lower milk yield that usually result in a concentration effect on fat. The lower percentage of saturated fatty acids as well as the higher proportion of MUFA, polyunsaturated fatty acids (PUFA) and RA found in EX milk was an expected result due the longer time spent on pasture by EX in comparison with SI cows (Elgersma, 2015). Feeding system affected also OBCFAs that, except for 17:0, were lower in milk from extensive system. It is known that OBCFAs in ruminant milk and meat mainly arise from rumen microflora. As reviewed by Vlaeminck *et al*. (2006), OBCFAs are usually found in greater quantity in the products of animal raised in a feeding system characterized by a high forage:concentrate ratio (F:C). However, the response in terms of FA composition to the variation of F:C ratio is variable, likely due to changes in the rumen fermentations that lead to a different availability of OBCFAs precursors. In addition, similarly to our result, Cozma *et al*. (2017) found a lower proportion OBCFAs in cow milk during the grazing season in comparison with the winter diet, mainly based on hay and concentrate. Authors ascribed the causes of this result to the inhibitory effect of PUFA, provided in high amount by pasture, on the activity of rumen bacteria.

In our experimental conditions, we found a significant interaction between *SCD* genotype and feeding system on 4:0 to 12:0 FAs and on *t*11 18:1. In particular, we found that in the extensive system, where pasture was the main available feed, all these FAs had an evident decreasing trend from

g.10329CC (p.293AA) to g.10329TT (p.293VV) cows, with intermediate values for the heterozygous cows. No similar findings have been reported in literature on 4:0 to 12:0, as effect of *SCD*; on the contrary, Duchemin *et al.* (2013) found significant lower levels of 8:0, 10:0 and 12:0 in p.293VV cows, similarly to Schennink *et al.* (2008) for 10:0 and 12:0. Duchemin *et al.* (2013) studied the effects of season on milk fat composition in different *SCD* genotypes. The differences between seasons reported by these authors, with pasture being available only during summer, could be assimilated to the differences between the two feeding systems used in our experimental conditions. They found a significant *SCD* genotype by season interaction for *t*11 18:1 that, coherently with our results, was negatively associated to p.293V variant. This effect of *SCD* on *t*11 18:1 was significantly more evident during summer, concurrently with pasture availability. The authors suggest that this interaction could be due to scaling of genotype effect, rather than to re-ranking.

In conclusion, a feeding trial was conducted on Modicana dairy cows homogeneous for genotypes at the *DGAT1* and *ABCG2* loci but with three different genotype at the *SCD* locus. Cows were reared in two traditional EX and SI feeding systems, characterized by a different use of pasture (8h *v.* 2 h/day). The feeding system has proved to be the parameter that has most influenced milk production and composition. Our results do not confirm what reported in literature as regard the effect of the *SCD* polymorphism (g.10329C > T, p.A293V) on milk FA composition. Moreover, our results suggest that when the interaction between *SCD* genotypes and feeding systems on the milk FAs composition was considered, percentage of 4:0, 6:0, 8:0, 10:0, 12:0 and *t*11 18:1 gradually decreased from g.10329CC to heterozygous to g.10329TT genotype in the milk from cows reared in the extensive system. The same trend was not found in the SI system. This finding might be explained assuming that the feeding system, and in particular the use of high amount of pasture, could have resized the *SCD* polymorphism effects due to the different FAs composition present in the diet.

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Declaration of interest

The authors declare no conflicts of interest.

Ethics statement

All the animals were reared under real commercial farm conditions. Therefore, no pain, suffering, distress and lasting harm was caused to the animals involved in the present study. Blood samples used for the analysis of genetic polymorphisms were collected by authorized personnel during the periodic veterinary control.

Software and data repository resources

The authors declare that none of the data or model in the present paper are deposited in an official repository.

Supplementary material

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References

Ates A, Hosturk GT, Akis I, Gursel FE, Yardibi H and Öztabak K 2014. Genotype and allele frequencies of polymorphisms in *ABCG2*, PPARGC1A and OLR1 genes in indigenous cattle breeds Turkey. Acta Veterinaria 64, 73–80.

Carvajal AM, Huircan P, Dezamour JM, Subiabre I, Kerr B, Morales R and Ungerfeld EM 2016. Milk fatty acid profile is modulated by *DGAT1* and *SCD*1 genotypes in dairy cattle on pasture and strategic supplementation. Genetics and Molecular Research 15, 15027057.

Cecchinato A, Ribeca C, Maurmayr A, Penasa M, De Marchi M, Macciotta NP, Mele M, Secchiari P, Pagnacco G and Bittante G 2012. Short communication: effects of β-lactoglobulin, stearoyl-coenzyme Adesaturase 1, and sterol regulatory element binding protein gene allelic variants on milk production, composition, acidity, and coagulation properties of Brown Swiss cows. Journal of Dairy Science 95, 450–454.

Ceriotti G, Marletta D, Caroli A and Erhardt G 2004. Milk protein loci polymorphism in taurine (*Bos taurus*) and zebu (*Bos indicus*) populations bred in hot climate. Journal of Animal Breeding and Genetics 121, 404–415.

Chilliard Y, Glasser F, Ferlay A, Bernard L, Rouel J and Doreau M 2007. Diet, rumen biohydrogenation and nutritional quality of cow and goat milk fat. European Journal of Lipid Science and Technology 109, 828–855.

Christie WW 1982. A simple procedure for rapid transmethylation of glycerolipids and cholesteryl esters. Journal of Lipids Research 23, 1072–1075.

Cohen-Zinder M, Seroussi E, Larkin DM, Loor JJ, Evertsvan der Wind A, Lee JH, Drackley JK, Band MR, Hernandez AG, Shani M, Lewin HA, Weller JI and Ron M 2005. Identification of a missense mutation in the bovine *ABCG2* gene with a major effect on the QTL on chromosome 6 affecting milk yield and composition in Holstein cattle. Genome Research 15, 936–944.

Conte G, Mele M, Chessa S, Castiglioni B, Serra A, Pagnacco G and Secchiari P 2010. Diacylglycerol acyltransferase 1, stearoyl-CoA desaturase 1, and sterol regulatory element binding protein 1 gene polymorphisms and milk fatty acid composition in Italian Brown cattle. Journal of Dairy Science 93, 753–763.

Cozma A, Martin B, Ciri C, Verdier-Metz I, Agabriel J and Ferlay A 2017. Influence of the calf presence during milking on dairy performance, milk fatty acid composition, lipolysis and cheese composition in Salers cows during winter and grazing seasons. Journal of Animal Physiology and Animal Nutrition 101, 949–963.

Duchemin S, Bovenhuis H, Stoop WM, Bouwman AC, van Arendonk JAM and Visker MH 2013. Genetic correlation between composition of bovine milk fat in winter and summer, and *DGAT1* and *SCD1* by season interactions. Journal of Dairy Science 96, 592–604.

Elgersma A 2015. Grazing increases the unsaturated fatty acid concentration of milk from grass-fed cows: a review of the contributing factors, challenges and future perspectives. European Journal of Lipid Science and Technology 117, 1345–1369.

Feng S, Salter AM, Parr T and Garnsworthy PC 2007. Extraction and Quantitative Analysis of Stearoyl-Coenzyme A Desaturase mRNA from Dairy Cow Milk Somatic Cells. Journal of Dairy Science 90, 4128–4136.

Garnsworthy PC, Feng S, Lock AL and Royal MD 2010. Short communication: heritability of milk fatty acid composition and stearoyl-CoA desaturase indices in dairy cows. Journal of Dairy Science 93, 1743–1748.

Gautier M, Capitan A, Fritz S, Eggen A, Boichard D and Druet T 2007. Characterization of the DGAT-1 K232A and variable number of tandem repeat polymorphism in French dairy cattle. Journal of Dairy Science 90, 2980–2988.

Grisart B, Coppieters W, Farnir F, Karim L, Ford C, Berzi P, Cambisano N, Mni M, Reid S, Simon P, Spelman R, Georges M and Snell R 2002. Positional candidate cloning of a QTL in dairy cattle: identification of a missense mutation in the bovine *DGAT1* gene with major effect on milk yield and composition. Genome Research 12, 222–231.

Guastella AM, Sorbolini S, Zuccaro A, Pintus E, Bordonaro S, Marletta D and Macciotta NPP 2011. Melanocortin 1 receptor (MC1R) gene polymorphisms in three Italian cattle breeds. Animal Production Science 51, 1039–1043.

Juhlin J, Fikse WF, Pickova J and Lunden A 2012. Association of DGAT1 genotype, fatty acid composition, and concentration of copper in milk with spontaneous oxidized flavour. Journal of Dairy Science 95, 4610–4617.

Kaupe B, Winter A, Fries R and Erhardt G 2004. *DGAT1* polymorphism in *Bos indicus* and *Bos taurus* cattle breeds. Journal of Dairy Research 71, 182–187.

Kelsey JA, Corl BA, Collier RJ and Bauman DE 2003. The effect of breed, parity, and stage of lactation on conjugated linoleic acid (CLA) in milk fat from dairy cows. Journal of Dairy Science 86, 2588–2597.

Komisarek J and Dorynek Z 2009. Effect of *ABCG2*, PPARGC1A, OLR1 and *SCD*1 gene polymorphism on estimated breeding values for functional and production traits in Polish Holstein-Friesian bulls. Journal of Applied Genetics 50, 125–132.

Komisarek J, Michalak A and Walendowska A 2011. The effects of polymorphisms in *DGAT1*, GH and GHR genes on reproduction and production traits in Jersey cows. Animal Science Papers Reports 29, 29–36.

Kgwatalala PM, Ibeagha-Awemu EM, Mustafa AF and Zhao X 2009. Influence of stearoyl-coenzyme A desaturase 1 genotype and stage of lactation on fatty acid composition of Canadian Jersey cows. Journal of Dairy Science 92, 1220–1228.

Lillehammer M, Hayes BJ, Meuwissen TH and Goddard ME 2009. Gene by environment interactions for production traits in Australian dairy cattle. Journal of Dairy Science 92, 4008–4017.

Luna P, Juàrez M and De La Fuenta MA 2005. Validation of a rapid milk fat separation method to determine the fatty acid profile by gas chromatography. Journal of Dairy Science 88, 3377–3381.

Macciotta NP, Mele M, Conte G, Serra A, Cassandro M, Dal Zotto R, Cappio Borlino A, Pagnacco G and Secchiari P 2008. Association between a polymorphism at the stearoyl coa desaturase locus and milk production traits in italian holsteins. Journal of Dairy Science 91, 3184–3189.

Marchitelli C, Contarini G, De Matteis G, Crisà A, Pariset L, Scatà MC, Catillo G, Napolitano F and Moioli B 2013. Milk fatty acid variability: effect of some candidate genes involved in lipid synthesis. Journal of Dairy Research 80, 165–173.

McDowell LR 1996. Feeding mineral to cattle on pasture. Animal Feed Science and Tecnology 60, 247–271.

Milanesi E, Nicoloso L and Crepaldi P 2008. Stearoyl CoA desaturase (*SCD*) gene polymorphisms in Italian cattle breeds. Journal of Animal Breeding and Genetics 125, 63–67.

Moioli B, Contarini G, Avalli A, Catillo G, Orrù L, De Matteis G, Masoero G and Napolitano F 2007. Effect of stearoyl-coenzyme A desaturase polymorphism on fatty acid composition of milk. Journal of Dairy Science 90, 3553–3558.

Mousavizadeh SA, Salehi A, Aminafshar M, Sayyadnejad M and Nazemshirazi MH 2013. Novel SNPs of the *ABCG2* gene and their associations with milk production traits in Iranian Holstein bulls. Journal of Agricultural Science and Technology 15, 1145–1151.

Näslund J, Fikse WF, Pielberg GR and Lundén A 2008. Frequency and effect of the bovine acyl-CoA: diacylglycerol acyl transferase 1 (*DGAT1*) K232A polymorphism in Swedish dairy cattle. Journal of Dairy Science 91, 2127–2134.

Ogorevc J, Kunej T, Razpet A and Dovc P 2009. Database of cattle candidate genes and genetic markers for milk production and mastitis. Animal Genetics 40, 832–851.

Pegolo S, Cecchinato A, Mele M, Conte G, Schiavon S and Bittante G 2016. Effects of candidate gene polymorphisms on the detailed fatty acids profile determined by gas chromatography in bovine milk. Journal of Dairy Science 99, 4558–4573.

Scotti E, Fontanesi L, Schiavini F, La Mattina V, Bagnato A and Russo V 2010. *DGAT1* p.K232A polymorphism in dairy and dual purpose Italian cattle breeds. Italian Journal of Animal Science 9, 79–82.

Sharma A, Tiwari M, Pal Singh S, Sharma D, Kumar S, Sharma A and Verma AK 2016. Study of *ABCG2* gene polymorphism in Sahiwal and Hariana Cattle by PstI/PCR-RFLP assay. Journal of Animal Research 6, 475–477.

Schennink A, Heck JM, Bovenhuis H, Visker MH, van Valenberg HJ and van Arendonk JA 2008. Milk unsaturation: genetic parameters and effects of stearoyl-CoA desaturase (*SCD*1) and acyl CoA: diacylglycerol acyltransferase 1 (*DGAT1*). Journal of Dairy Science 91, 2135–2143.

Taniguchi M, Utsugi T, Oyama K, Mannen H, Kobayashi M, Tanabe Y, Ogino A and Tsuji S 2004. Genotype of stearoyl-coA desaturase is associated with fatty acid composition in Japanese Black cattle. Mammalian Genome 15, 142–148.

Thompson J, Higgins D and Gibson T 1994. Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position – specific gap penalties and weight matrix choice. Nucleic Acids Research 22, 4673–4680.

Vlaeminck B, Fievez V, Cabrita ARJ, Fonseca AJM and Dewhurst RJ 2006. Factors affecting odd- and branched-chain fatty acids in milk: a review. Animal Feed Science and Technology 131, 389–417.