Stearoyl-Coenzyme A Desaturase Gene Polymorphism and Milk Fatty Acid Composition in Italian Holsteins

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ABSTRACT

Milk fatty acid composition is a parameter of great interest for evaluation of nutritional quality of milk. Stearoyl-CoA desaturase (SCD) is a key enzyme in mammary lipid metabolism because it is able to add a double bond in the *cis* Δ^9 -position in a large spectrum of medium- and long-chain fatty acids. A polymorphism with 2 alleles (A and V) in the fifth exon of the *SCD* gene has been reported. The effect of *SCD* genotype on individual milk fatty acid composition and on *cis*-9 unsaturated/saturated fatty acid ratios of 297 Holstein Italian Friesian cows was investigated in this paper. The *SCD* genotypes were determined by using a single strand conformation polymorphism method. Relative frequencies of *SCD* genotypes were 27, 60, and 13% for AA, AV, and VV, respectively. Milk of AA cows had a greater content of *cis*-9 C18:1 and total monounsaturated fatty acids and a higher C14:1/C14 ratio than did milk of VV cows. The relative contribution of *SCD* genotype to variation of monounsaturated fatty acids, *cis*-9 C18:1, and *cis*-9 C14:1 was 5, 4, and 7.7%, respectively. No significant differences were detected between *SCD* genotypes in the milk content of *cis*-9, *trans*-11 C18:2. Results of the present work provide some indication of an association between *SCD* locus and the fatty acid profile in the examined sample of Italian Holsteins, thus suggesting a possible role of this gene in the genetic variation of milk nutritional properties.

Key words: stearoyl-CoA desaturase polymorphism, conjugated linoleic acid, monounsaturated fatty acid

INTRODUCTION

The fatty acid (FA) fraction of ruminant's milk contains several compounds of great interest for human

health, such as monounsaturated FA (mainly oleic acid) and conjugated linoleic acids (CLA). Studies aimed at finding efficient strategies to improve the nutritional quality of milk concluded that feeding supplementation is the most efficient way to modify milk FA (Palmquist et al., 1993; Jenkins and McGuire, 2006), even if a recent study suggested that the genetic improvement of the nutritional quality of milk based on FA profile may be possible (Soyeurt et al., 2006). However, traditional selection programs based on performance recording and prediction of breeding values could be constrained by the high costs for the measurement of phenotypes. The search for loci affecting FA profiles and their use in marker assisted selection programs offer an alternative to modify nutritional qualities of milk through genetic selection.

Among the several enzymes known to affect the lipid metabolism of the mammary gland, the stearoyl-CoA desaturase enzyme (SCD; also known as Δ^9 -desaturase) plays a key role because it introduces a double bond at the Δ^9 -position in a large spectrum of FA (Ntambi, 1999; Ntambi and Miyazaki, 2004). Its most important substrates are acyl-CoA of C14, C16, C18, and *trans*-11 C18:1 (known as vaccenic acid), which are converted into C14:1 n-5, C16:1 n-7, *cis*-9 C18:1, and *cis*-9, *trans*-11 C18:2, respectively (Corl et al., 2001), the latter being a CLA isomer of great interest due to its antiatherogenic, anticarcinogenic, and immunomodulating properties (Pariza, 1999). More than 70% of the *cis*-9, *trans*-11 CLA of ruminant's milk is produced in the mammary tissue by the activity of SCD (Bauman et al., 2006).

The *SCD* locus has been mapped on the bovine chromosome 26. It is 17,088 bp long with an open reading frame of 1,080 bp and codes for 359 amino acids. Three single nucleotide polymorphisms (SNP) in complete linkage disequilibrium and that result in 2 haplotypes have been detected in the fifth exon (Taniguchi et al., 2004). The third SNP causes the substitution of valine

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Haplotype V:
gtactacaaacctgggtgctctgtgtgtgcttcacctgcccacactcgtgccatggtatctgtggatgaacgttcaaacagcctgtttttgccc
accttattccgttatgccctgggctcaacgtcacctggctggtgaatagtgctgcccatactgtatggataccgcccttatgacaagaccatcaacc
cccagagagaatattctgtttccctgggagctg
Haplotype A:
gtactacaaacctgggtgctctgtgtgtgcttcacctgcccacactcgtgccatggtatctgtggatgaacgttcaaacagcctgtttttgccc
caccttattccgttatgccctgggctcaacgtcacctggctggtgaatagtgctgcccatactgtatggataccgcccttatgacaagaccatcaac
ccccgagagagaatattctgtttccctgggagctgtg

Figure 1. Bovine stearoyl-CoA desaturase (*SCD*) polymorphism. The gray boxes show the nucleotide substitution that causes the amino acid replacement valine 293→alanine 293.

(allele V) with alanine (allele A) on the 293rd residue (Figure 1). Because valine is highly conserved across mammals, it is considered the ancestral amino acid in that position (Taniguchi et al., 2004). The *SCD* polymorphism has been found in Holstein Friesian, Jersey, Brown Swiss, and Japanese Black cattle breeds (Medrano et al., 1999; Taniguchi et al., 2004).

On the basis of the *SCD* gene function and of the relationships between its genetic polymorphism and FA composition of carcass fat recently observed in cattle (Taniguchi et al., 2004), this locus may be a possible candidate gene able to explain a portion of the genetic variability of milk FA content in cows. The objective of this study was the investigation of possible associations between *SCD* polymorphisms and milk FA composition in Italian Holsteins.

MATERIALS AND METHODS

Animals

The analysis was carried out on 297 Italian Holstein Friesian cows sampled from 21 half-sib families (14 ± 2 daughters per bull) and distributed in 34 herds (9 ± 3 cows per herd) located in the northeast of Italy.

DNA Extraction and Amplification

Genomic DNA was extracted from milk somatic cells with the GFX Genomic Blood DNA Purification Kit (Amersham Biosciences, Uppsala, Sweden) following the supplier's protocol with some modifications. Briefly, 900 μ L of RBC lysis solution were added to 300 μ L of milk in a 1.5-mL microcentrifuge tube, incubated at room temperature, and then centrifuged at $11,300 \times g$

for 1 min. The supernatant was removed, and the residual fluid was vortexed and then mixed with 500 μ L of extraction solution and incubated at room temperature. The mixture was then transferred to the GFX column and centrifuged at $4,300 \times g$ for 2 min. The DNA was removed with an extraction solution (buffered solution containing chaotrope and detergent) and centrifuged again at $4,300 \times g$ for 2 min. A second washing with 500 μ L of wash solution (Tris-EDTA buffer added with absolute ethanol) was performed, followed by a full speed centrifuge ($11,300 \times g$) for 4 min. The GFX column was transferred into a fresh 1.5-mL tube for the elution. One hundred microliters of 70°C preheated double-distilled water were added directly to the fiberglass matrix of the column. After 1 min of incubation at room temperature, the tube was centrifuged at $4,300 \times g$ for 2 min to collect the purified DNA.

The fifth exon region of the *SCD* gene was amplified by PCR (GeneAmp PCR 9600; Perkin Elmer Life Sciences). Specific primers were designed on the basis of the bovine *SCD* gene sequence (Medrano et al., 2003, accession no. AY241932) at GenBank (Figure 2):

SCD-9848F: 5'-CAGTCCTTGCTCCACCACTT-3';

SCD-10572R: 5'-AGCATTTGTGGCTTGCTCTT-3';

SCD-9961F: 5'-CCCATTCGCTCTTGTCTGT-3';

SCD-10355R: 5'-GTCTTGCTGTGGACTGCTGA-3'.

The DNA was preheated at 94°C for 5 min and then amplified with 30 cycles of 94°C for 45 s, 56°C for 45 s, and 72°C for 1 min. The final cycle had an extension time of 7 min at 72°C. The PCR reaction was performed using a mix of 12.5 μ L of 2 $\times$ PCR Master Mix (Fer-

9721 gaaaggaagcccaaggcagaa cagaaaac ccggaaggagaact cagtctgtaatttg
 9781 gaccctgtctatttccctgaagtagtaaaagagagcaatggat ttagagt caggagatga

SCD-9848F →

9841 aacattc cagtccttgctccaccacttc ctggtagtgtgaccc tagaaa agt cat ttaac
 9901 ccctcat tacctcatctcagaagta tga taa taa cactgt att ctcagaaa ttt ccttg

SCD-9961F →

9961 cccattegtctcttgttctgttg cctgccacactgtgtctct cagtgt agaaaggga cagcc
 10021 ccgcctgtgagagtggaaaat caggtagggt ctcagcgtccctct cca ctcactgagcc
 10081 tgtgatctctcaatgcagg **tac tac aaa cctgggtgcctgtttgttg tgc ttc atc ctg cc**
 10141 **cacactcgtgccatgggtatctgtgggatgaaacgttt caaaac agc ctg ttt ttt gcc ac**
 10201 **ctta ttc cgt tat gcc ctt ggg ctc aac gtc acc tgg ctggtgaat agt gct gcc cat at**
 10261 **gtatgga tac cgc cct tat gac aag acc atc aac ccc cgagagaat att ctggtt tcc ct**

SCD-10355R ←

10321 **gggagctgcgggt** aag tcagcagtc cacagcaagacc acgtct agt ggt ctgctgcttag
 10381 ggtattaggt tacgtgccagaaaa ctatgat tta cctgtt tta tga ccc ctc tccata tg
 10441 tcattccactataaaa ctaagggacagt att agaaaa ccc ttgaaagtta agcaacaagt

← **SCD-10572R**

10501 cctatgt aaagagaaaagggtgaaaaat gacaatgccttta at tccagggtt aaagacaa

SCD-10572R

10561 gccacaaatgct atgt atgatgaacccctttgggctgttctct tccagtcattct ctgat

Figure 2. Positions of stearoyl-CoA desaturase (*SCD*) primers. Bold type shows the *SCD* gene fifth exon. The primers were designed by Medrano et al. (2003).

mentas International Inc., Burlington, Canada; *Taq* DNA polymerase 0.05 units/ μ L, $MgCl_2$, 4 mM, and dNTP, 0.4 mM of each), 0.5 μ L of 10 pmol/ μ L primer forward, 0.5 μ L of 10 pmol/ μ L reverse primer, 250 ng of DNA and ddH₂O up to 25 μ L. Among the several PCR products, the 725-bp fragment obtained using *SCD*-9848F and *SCD*-10572R primers was retained for the genotyping (Figure 3).

PCR-Single Strand Conformation Polymorphism Analysis

The *SCD* genotypes were assessed analyzing the DNA fragment containing the polymorphisms with an adapted single strand conformation polymorphism (SSCP) method (Ceriotti et al., 2004): 6 μ L of PCR product were added to 8 μ L of denaturing solution (0.025% of xylene-cyanol, 0.025% of bromophenol blue, and 0.02 M EDTA in deionized formamide). After heat denaturation (95°C for 8 min), samples were immediately chilled on ice and then run (16 h, 280 V, 5°C) on 8% acrylamide:bis-acrylamide gels (37:1) with 1% glycerol in 0.5 $\times$ Tris-borate-EDTA buffer. The SSCP method was preferred due to its capability to detect other possible modifications in the fragment analyzed.

Figure 3. The PCR products: lane 1 and 6- GeneRuler 1 kb DNA ladder (Fermentas International Inc., Burlington, Canada); lane 2- *SCD*-9848F/*SCD*-10355R product (508 bp); lane 3 *SCD*-9848F/*SCD*-10572R product (725 bp); lane 4 *SCD*-9961F/*SCD*-10572R product (612 bp); lane 5 *SCD*-9961F/*SCD*-10355R product (395 bp).

DNA Sequencing

The DNA samples with different SSCP gel patterns were further sequenced by PRIMM Srl (Milan, Italy). One sample for every assumed genotype was randomly chosen. Primers used for sequencing were the same of the PCR-SSCP analysis. Nucleotide sequences were analyzed with Chromas 2.24 software (Technelysium Pty Ltd.).

Milk Fat Analysis

Individual milk samples were taken at the morning milking and stored at -20°C . One milk sample was taken per animal. Milk fat was extracted following the Rose-Gottlieb method (AOAC, 1990) modified by Secchiari et al. (2003). Two grams of milk were mixed with 0.4 mL of ammonia 25%, 1 mL of ethyl alcohol 95%, and 5 mL of hexane, vortexed, and centrifuged at $1,600 \times g$ and 4°C . The upper layer was collected, and a second extraction with 1 mL of ethyl alcohol 95% and 5 mL of hexane was performed. A third extraction was made using 5 mL of hexane. The extracted fat was dried, weighed, and finally dissolved in hexane. Methyl esters of medium- and long-chain fatty acids were prepared by Christie's alkali catalyzed transmethylation procedure (1982) with nonadecanoic acid methyl ester (Sigma Chemical Co., St. Louis, MO) as the internal standard. Medium- and long-chain FA composition was determined by gas chromatography using a ThermoQuest (Milan, Italy) gas-chromatograph equipped with an FID and a high polar fused silica capillary column (Chrompack CP-Sil 88 Varian, Middelburg, the Netherlands; $100 \text{ m} \times 0.25 \text{ mm i.d.}$; film thickness $0.20 \mu\text{m}$). Helium was used as the carrier gas at a flow of 1 mL/min . The split ratio was 1:100. An aliquot of the sample was injected under the following GC conditions: the oven temperature was programmed at 120°C and held for 1 min, then increased to 180°C at a rate of 5°C/min , held for 18 min, increased to 200°C at 2°C/min , held for 1 min, increased to 230°C at a rate of 2°C/min and held for 19 min. The injector temperature was set at 270°C , whereas the detector temperature was set at 300°C . *Cis* and *trans* C18:1 isomers were determined on a second aliquot of the same sample at 175°C (isothermal step) for 65 min using the same capillary column. Individual FA methyl esters were identified by comparing them to a standard mixture of 37 Component FAME Mix (Supelco, Bellefonte, PA). The standards PUFA-2, non-conjugated C18:2 isomer mixture, individuals *cis*-5,8,11,14,17 C20:5, *cis*-4,7,10,13,16,19 C22:6 (Supelco), *cis*-6,9,12 C18:3 and *cis*-9,12,15 C18:3 (Matreya Inc., Pleasant Gap, PA) were used to identify polyunsaturated FA. The identification of C18:1 isomers was based on commercial standard mixtures (Supelco) and pub-

lished isomeric profiles (Wolff and Bayard, 1995). All the methods that use peak normalization and that express results as a relative percentage of the area of the analyzed peaks are subject to overestimation because of small peak areas not considered. To avoid this problem, a nonadecanoic acid as internal standard was used and all milk FA compositions were expressed as g/100 g of fat.

Ratios of Milk Fatty Acids

The extent of FA desaturation was determined by calculating the ratio of *cis*-9 unsaturated to *cis*-9 unsaturated + saturated for specific FA (Sol-Morales et al., 2000; Palmquist et al., 2004). The following ratios were calculated: *cis*-9 C14:1 to *cis*-9 C14:1 + C14:0 (C14:1/C14); *cis*-9 C16:1 to *cis*-9 C16:1 + C16:0 (C16:1/C16); *cis*-9 C18:1 to *cis*-9 C18:1 + C18:0 (C18:1/C18); *cis*-9, *trans*-11 CLA to *cis*-9, *trans*-11 CLA + *trans*-11 C18:1 (CLA/*trans*-11 C18:1).

Moreover, a general index of desaturation (**DI**) was calculated (adapted from Malau-Aduli et al., 1997):

$$\text{DI} = 100 \times [(cis-9 \text{ C14:1} + cis-9 \text{ C16:1} + cis-9 \text{ C18:1} + cis-9, trans-11 \text{ CLA}) / (cis-9 \text{ C14:1} + cis-9 \text{ C16:1} + cis-9 \text{ C18:1} + cis-9, trans-11 \text{ CLA} + \text{C14:0} + \text{C16:0} + \text{C18:0} + trans-11 \text{ C18:1})].$$

Statistical Analysis

Data were analyzed with the following mixed linear model (SAS, 1999):

$$y_{jklmno} = \mu + \text{HERD}_j + \text{PARITY}_k + \text{DIM}_l + \text{SEASON}_m + \text{SCD}_n + \text{SIRE}_o + \epsilon_{jklmno} \quad [1]$$

where y_{jklmno} = dependent variable (milk fatty acids as g/100 g of lipids, and FA ratios); μ = overall mean; HERD_j = fixed effect of the j th herd (34); PARITY_k = fixed effect of the k th parity (first, second, $\geq$ third); DIM_l = fixed effect of the l th days in milking interval (<100 , 100 to 200, >200); SEASON_m = fixed effect of the m th calving season (winter, spring, summer, autumn); SCD_n = fixed effect of the n th *SCD* genotype (AA, AV, VV); SIRE_o = random effect of the o th sire (21); and ϵ_{jklmno} = random residual. Results on the *SCD* genotype effects were presented as least square means $\pm$ SE, and linear contrasts were tested between AA and AV genotypes and between AV and VV genotypes.

The average gene substitution effect for the *SCD* locus on milk FA content was calculated according to

Figure 4. The PCR-single strand conformation polymorphism (SSCP) analysis for the fifth exon of the stearoyl-CoA desaturase (*SCD*) gene polymorphism. The white and the black dots indicate the occurrence of A and V, respectively.

Falconer and McKay (1996). The contribution of the *SCD* locus to the variability of the traits considered was assessed by calculating the reduction of residual variance observed in the full model [1], in comparison with a reduced model that did not include the *SCD* genotype as fixed factor (Neter et al., 1996).

RESULTS AND DISCUSSION

Three different patterns were detected by the PCR-SSCP analysis of the DNA fragment containing the polymorphism of the *SCD* gene (Figure 4). The occurrence of a single lower or upper band was considered as evidence for a homozygous AA or VV, respectively, whereas both bands were regarded as an evidence for a heterozygous AV. This genotype assignment was confirmed by nucleotide sequencing. The different SSCP patterns corresponded to the third SNP (Figure 5): alleles V and A match the upper and the lower band, respectively.

Frequencies of *SCD* genotypes observed in this sample of Italian Friesian cows are reported in Table 1. Genotypes were not in Hardy-Weinberg equilibrium ($P < 0.05$); similar figures reported by Taniguchi et al. (2004) were ascribed to the sampling effect and to the selection pressure for other traits. Compared with the V allele, the higher frequency of the A allele (0.57 vs. 0.43, for alleles A and V, respectively) is in agreement with results reported by the above-mentioned study (0.59 vs. 0.41 for alleles A and V, respectively).

Stage of lactation affected significantly monounsaturated FA (MUFA), *cis*-9 C18:1, and *cis*-9 C14:1 and C14:0 contents. In the first 100 d of lactation, higher *cis*-9 C18:1 and lower C14:0 and *cis*-9 C14:1 contents were observed in comparison with later stages of lactation (Table 2), probably due to the mobilization of body fat reserves and to the inhibition of mammary lipogenic enzymes that occur in animals with negative energy

Figure 5. Nucleotide sequence results. The figure shows a region around the third single nucleotide polymorphism of a heterozygous sample, which is indicated by the 2 arrows.

balance (Kay et al., 2005). Parity and calving season did not affect significantly the variables considered.

The *SCD* genotype affected significantly ($P \leq 0.01$) *cis*-9 C18:1, *cis*-9 C14:1, and total MUFA contents (Table 3). Particularly, the AA genotype was associated with 9.3, 37.9, and 11.7% more MUFA, *cis*-9 C14:1 and *cis*-9 C18:1, respectively, when compared with VV.

The *SCD* average gene substitution effect was 0.83, 0.93, and 0.108% for MUFA, *cis*-9 C18:1, and *cis*-9 C14:1 content, respectively; the AA genotype showed 1.77, 1.93, and 0.22 higher MUFA, *cis*-9 C18:1, and *cis*-9 C14:1 contents than the genotype VV (Table 3), respectively. These results are in agreement with those reported by Taniguchi et al. (2004) on FA composition of carcass fat in cattle.

The relationship between *SCD* polymorphism and milk *cis*-9, *trans*-11 CLA content is of particular inter-

Table 1. Distribution of stearoyl-CoA desaturase (*SCD*) genotypes in the 297 Italian Holsteins considered

Genotype	Absolute frequencies (n)	Relative frequencies (%)
AA	80	27
AV	179	60
VV	38	13
Total	297	100

Table 2. Least squares means of selected measures of milk fatty acid content (g/100 g of total lipids) for different DIM intervals

Fatty acid	DIM					
	<100		100 to 200		>200	
	Mean	SE	Mean	SE	Mean	SE
C14:0	8.04 ^A	0.24	9.33 ^B	0.18	9.38 ^B	0.28
C16:0	23.70	0.56	25.09	0.63	24.02	0.44
C18:0	9.21	0.33	8.43	0.39	8.63	0.25
<i>Trans</i> -11 C18:1	0.73	0.03	0.75	0.05	0.80	0.02
<i>Cis</i> -9 C14:1	0.56 ^A	0.03	0.66 ^{AB}	0.04	0.79 ^B	0.02
<i>Cis</i> -9 C16:1	1.09	0.04	1.18	0.06	1.25	0.05
<i>Cis</i> -9 C18:1	18.99 ^a	0.44	17.88 ^b	0.54	17.86 ^b	0.35
<i>Cis</i> -9, <i>trans</i> -11 CLA ¹	0.37	0.03	0.34	0.04	0.40	0.03
MUFA ²	21.58 ^a	0.42	20.08 ^{ab}	0.48	19.33 ^b	0.40

^{A,B}Means within rows with different superscripts differ ($P < 0.01$).

^{a,b}Means within rows with different superscripts differ ($P < 0.05$).

¹CLA = conjugated linoleic acid.

²MUFA = monounsaturated fatty acids.

est. Milk *cis*-9, *trans*-11 CLA content is characterized by individual variation (up to 8-fold) that can be observed in cows fed the same diet (Bauman et al., 2001; Chilliard et al., 2001; White et al., 2001; Secchiari et al., 2003). Therefore, an important role of the genetic background of animals could be hypothesized. In our study, the AA genotype was associated with 12% more *cis*-9, *trans*-11 CLA when compared with VV; however, this difference did not reach the level of significance ($P = 0.19$), perhaps due to the small size of the sampled population.

In terms of explained variance, the contribution of *SCD* genotype to MUFA variation in milk fat was quite low (5%), in agreement with results reported for intramuscular fat in cattle (Taniguchi et al., 2004). Of the same magnitude are contributions of the *SCD* genotype to *cis*-9 C18:1, and *cis*-9 C14:1 variation (4 and 7.7%,

respectively). These results suggest a reproducible and ubiquitous effect of the amino acid substitution. The contribution of *SCD* genotype to the total variation of other FA profiles was nonsignificant and less than 1%.

Sire effects accounted for 6, 5, and 8% of the total variance for content of *cis*-9 C18:1, and *cis*-9 C14:1, and MUFA, respectively. These values are indicative of an appreciable additive genetic effect on the variation in milk FA composition.

The analysis of desaturation activity indicators highlighted a significant effect of the *SCD* polymorphism only on the C14:1/C14 ratio, with homozygous AA cows having the highest value (Table 4). The absence of a relationship between *SCD* genotype and the other FA ratios could be ascribed to the wide range of factors influencing desaturation activity of the mammary gland such as differential uptake, use, and turnover of

Table 3. Least squares means of selected measures of milk fatty acid content (g/100 g of total lipids) for the 3 different stearoyl-CoA desaturase (*SCD*) genotypes

Fatty acid	<i>SCD</i> genotype					
	AA		AV		VV	
	Mean	SE	Mean	SE	Mean	SE
C14:0	8.49	0.25	8.85	0.17	8.88	0.31
C16:0	23.92	0.60	24.12	0.41	23.17	0.72
C18:0	8.70	0.35	8.91	0.24	8.58	0.42
<i>Trans</i> -11 C18:1	0.76	0.03	0.77	0.02	0.74	0.03
<i>Cis</i> -9 C14:1	0.80 ^A	0.04	0.68 ^B	0.03	0.58 ^B	0.05
<i>Cis</i> -9 C16:1	1.17	0.05	1.16	0.04	1.10	0.06
<i>Cis</i> -9 C18:1	18.43 ^A	0.44	17.68 ^B	0.30	16.50 ^B	0.53
<i>Cis</i> -9, <i>trans</i> -11 CLA ¹	0.37	0.01	0.36	0.01	0.33	0.02
MUFA ²	20.72 ^A	0.47	20.30 ^A	0.32	18.95 ^B	0.57

^{A,B}Means within rows with different superscripts differ ($P < 0.01$).

¹CLA = conjugated linoleic acid.

²MUFA = monounsaturated fatty acids.

Table 4. Least squares means of the fatty acid ratios for the 3 different stearoyl-CoA desaturase (*SCD*) genotypes

Fatty acid	<i>SCD</i> genotype					
	AA		AV		VV	
	Mean	SE	Mean	SE	Mean	SE
14:1/14 ¹	0.081 ^A	0.004	0.072 ^B	0.002	0.064 ^B	0.004
16:1/16 ²	0.047	0.002	0.048	0.001	0.044	0.002
18:1/18 ³	0.676	0.008	0.668	0.006	0.658	0.011
CLA/ <i>trans</i> -11 C18:1 ⁴	0.329	0.007	0.321	0.006	0.314	0.009
Desaturation index (DI) ⁵	0.331	0.007	0.321	0.005	0.311	0.009

^{A,B}Means within rows with different superscripts differ ($P < 0.01$).

¹14:1/14 = *cis*-9 C14:1/C14:0 + *cis*-9 C14:1.

²16:1/16 = *cis*-9 C16:1/C16:0 + *cis*-9 C16:1.

³18:1/18 = *cis*-9 C18:1/C18:0 + *cis*-9 C18:1.

⁴CLA/*trans*-11 C18:1 = *cis*-9, *trans*-11 C18:2/*trans*-11 C18:1 + *cis*-9, *trans*-11 C18:2.

⁵DI = (*cis*-9 C14:1 + *cis*-9 C16:1 + *cis*-9 C18:1 + *cis*-9, *trans*-11 C18:2) / (C14:0 + *cis*-9 C14:1 + C16:0 + *cis*-9 C16:1 + *cis*-9 C18:1 + C18:0 + *trans*-11 C18:1 + *cis*-9, *trans*-11 C18:2).

the different FA (Bernard et al., 2006). On the other hand, the C14:1/C14 ratio has been suggested as the best indicator for the *SCD* activity (Corl et al., 2001; Lock and Garnsworthy, 2003): C14:0 in milk fat derives almost exclusively from de novo synthesis in the mammary gland, and therefore, almost all the *cis*-9 C14:1 is likely to be synthesized by *SCD* (Bernard et al., 2006).

CONCLUSIONS

In the present study, a putative relationship between the genotype at the *SCD* locus and the content of some milk FA in Italian Holsteins was found. In particular, associations of *SCD* genotype with MUFA content and the C14:1/C14 ratio, an indicator of mammary desaturase activity, were indicated. Although the amount of MUFA variation explained by *SCD* genotype was low, *SCD* genotype may have a role in marker assisted selection programs aimed at improving the nutritional quality of milk. The absence of significant relationships between *SCD* polymorphism and milk *cis*-9, *trans*-11 CLA isomer content should be carefully considered. Reasons for the relevant individual variability of this trait are not fully understood, and further studies on mechanisms influencing the availability of *cis*-9, *trans*-11 CLA (or its precursors) from the rumen, on *SCD* gene expression regulation, and on differences in downstream factors that would affect interaction between enzyme and substrate (e.g., phosphorylation) are required (Bauman et al., 2003).

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