Effects of milk fat composition, *DGAT1*, and *SCD1* on fertility traits in Dutch Holstein cattle

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ABSTRACT

Recently, selective breeding was proposed as a means of changing the fatty acid composition of milk to improve its nutritional quality. Before implementing such breeding objectives, effects on other economically important traits should be investigated. The objectives of this study were to examine 1) the effect of milk fat composition, and 2) the effect of polymorphisms of *DGAT1* and *SCD1* genes on female fertility in commercial Dutch Holstein-Friesian cattle. Data on 1,745 first-lactation cows were analyzed by fitting linear mixed models. We found that higher concentrations of *trans* fatty acids within total milk fat negatively affected reproductive performance. Furthermore, results suggested a potential effect of the *DGAT1* polymorphism on nonreturn rates for insemination 28 and 56 d after the first service. Our results can be used to assess the correlated effects of breeding for improved milk fat composition on reproduction, thereby allowing for better evaluation of breeding programs before implementation.

Key words: dairy cattle, milk fat composition, *DGAT1*, *SCD1*

INTRODUCTION

Because of biotechnological advances and a more complete knowledge of the cattle genome, new opportunities exist to produce raw milk with specific characteristics (Boland et al., 2001; Creamer et al., 2002). By using selective breeding, different types of raw milk can be produced to meet specific consumer needs or industrial demands. Recently, the opportunities to use selective breeding to change the fatty acid (FA) composition of milk have been investigated extensively. Changing the milk FA profile can improve the nutritional quality of milk and thus can lead to improved dairy products for human health.

Selective breeding to change milk FA composition can take 2 primary directions. First, cows can be selected for an individual milk FA or for a group of milk FA, because several milk FA have recently been shown to have genetic variation within various US (Bobe et al., 2008), Belgian (Soyeurt et al., 2007, 2008), and Dutch (Stoop et al., 2008) dairy cattle breeds. Second, cows can be selected for particular genotypes. Two genes have recently been suggested as candidates: the gene coding for acyl-coenzyme A:diacylglycerol acyltransferase 1 (*DGAT1*) and the gene coding for stearoyl-coenzyme A desaturase 1 (*SCD1*). The *DGAT1* gene is located on chromosome 14 in cattle (Grisart et al., 2002; Winter et al., 2002) and plays a key role in triacylglycerol synthesis. A lysine-to-alanine polymorphism in *DGAT1* (**K232A**) has been shown to affect milk FA composition (Schennink et al., 2007) and to explain some of the genetic variation in the milk fat unsaturation indices in Dutch Holstein cows (Schennink et al., 2008). The *SCD1* gene, which codes for a key enzyme in the cellular biosynthesis of monounsaturated FA, is located on chromosome 26 in cattle (Chung et al., 2000). An alanine-to-valine polymorphism in *SCD1* (**A293V**) has been found to influence milk FA composition in various Italian breeds (Mele et al., 2007; Moioli et al., 2007; Macciotta et al., 2008) and in Dutch Holstein cows (Schennink et al., 2008).

Before selecting for specific milk FA or candidate genes, however, effects on other economically important traits should be investigated. A relevant trait to consider is the reproductive performance of cows, given its high economic importance to dairy owners and managers (Lehenbauer and Oltjen, 1998). Little (and contradictory) information is available on the effects of *DGAT1* variants on fertility traits (Ashwell et al., 2004; Kaupe et al., 2004; Wathes et al., 2008). Kaupe et al. (2007) found that the K allele had a negative effect on maternal nonreturn rate. Oikonomou et al. (2009), however, showed that the A allele increased the number of inseminations per conception and the presence of reproductive problems, and decreased the conception rate in the first 305 d of lactation. To our knowledge,

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estimates of the effects of milk FA composition and *SCD1* variants on the reproductive traits of dairy cattle are missing from the literature. The objectives of this study were to examine 1) the effect of milk fat composition, and 2) the effect of the *DGAT1* K232A polymorphism and the *SCD1* A293V polymorphism on fertility traits in commercial Holstein-Friesian dairy cattle in the Netherlands.

MATERIALS AND METHODS

Animals

We used data from the Dutch Milk Genomics Initiative, which focuses on the genetic background of detailed milk composition (Schennink et al., 2007; Stoop et al., 2007; Schopen et al., 2009). Data regarding 1,745 first-lactation cows distributed among 394 commercial herds throughout the Netherlands were collected. Participation invitations to the farms were based mainly on pedigree according to the database of the cooperative cattle improvement organization CRV (Arnhem, the Netherlands), which provided records on pedigree, yield, and reproduction. Each cow was more than 87.5% Holstein-Friesian. The cows descended from 1 of either 5 proven bulls (807 cows), 50 test bulls (771 cows), or 15 additional proven bulls (167 cows). Offspring from the last group of bulls were added to ensure sampling of at least 3 cows per herd. Further details on the animals used in this study are provided by Stoop et al. (2007).

Phenotypes

From the records on reproductive performance, the following 8 fertility traits were derived: interval (d) between the first and second calving (**CI**), interval (d) between calving and the first insemination (**DFS**), number of inseminations (**NINS**), nonreturn rate (0/1) for insemination 28 d after the first insemination (**NR28**), nonreturn rate (0/1) for insemination 56 d after the first insemination (**NR56**), nonreturn rate (0/1) for insemination 90 d after the first insemination (**NR90**), rate (0/1) of calving after the first insemination (**CFI**), and rate (0/1) of calving after any inseminations (**CR**). Records for CI were considered missing if an animal did not have a known next calving date, or if the CI was shorter than 300 d or longer than 800 d. Records for DFS were set to missing if the DFS was smaller than 20 d or greater than 300 d. The NINS was set to missing if its value was 0 or greater than 10. These criteria were applied to exclude unrealistic, unrepresentative, or erroneous records under Dutch conditions (Calus et al., 2005).

Cows were milked twice a day, and a morning milk sample from each cow was analyzed between February and March 2005. Each cow was between 66 and 282 d in lactation. Milk FA composition was measured by gas chromatography at the COKZ Laboratory (Leusden, the Netherlands) as described by Schennink et al. (2007). The FA were expressed as a proportion of total fat weight. Fatty acids included in this study were grouped according to their relevance to human health (German and Dillard, 2006). Fat and protein concentrations were measured by infrared spectroscopy with a Fourier-transform interferogram (MilkoScan FT 6000, Foss Electric, Hillerød, Denmark) at a Milk Control Station (Zutphen, the Netherlands). Furthermore, lactation yield records based on 305-d production for milk, protein, and fat were provided by CRV. In total, data regarding milk FA composition and the 5 production traits were missing for 19 animals.

Genotypes

Blood samples for DNA isolation were collected between April and June 2005. Genotyping of the *DGAT1* and the *SCD1* polymorphisms were performed as described by Schennink et al. (2007) and Schennink et al. (2008), respectively. Because either no DNA sample was available or the sample could not be genotyped unambiguously, the genotype for *DGAT1* was missing for 12 animals, and the genotype for *SCD1* was missing for 66 animals. When studying the effects of candidate gene polymorphisms, we also accounted for production traits (see the Statistical Analysis section below). To study the effects of the genes consistently, therefore, animals were excluded if no information was available on the 5 milk production traits.

Two data sets were formulated and used in this study. The first data set, which included 1,726 animals, was used to study the effects of milk FA composition and the *DGAT1* polymorphism on fertility traits. Descriptive statistics of FA and milk production traits of the 1,726 animals are provided in Table 1. The average 305-d milk yield was 7,623 kg, and 305-d protein and fat yields were 262 and 327 kg, respectively. Protein and fat percentages averaged 3.5 and 4.4%, respectively. The reproductive performance of the 1,726 cows in the first data set is described in Table 2. The average length of the calving interval was 411 d, and the mean interval between calving and the first insemination was approximately 90 d. Approximately 10% of the cows did not have a second calving. The second data set included 1,661 animals and was used to study the effects of the *SCD1* polymorphism; descriptive statistics for the second data set are not shown because they are similar to those of the first data set.

Table 1. Descriptive statistics of individual fatty acids, fatty acid groups, and production traits of 1,726 first-lactation Dutch Holstein-Friesian cows

Trait	Mean	Minimum	Maximum	SD
Fatty acid (% wt/wt)				
C4:0	3.51	2.44	4.57	0.28
C5 to C15 ¹	1.47	0.72	3.90	0.32
C6 to C12 ²	10.73	5.10	15.50	1.24
C14:0	11.62	6.17	14.94	0.93
C16:0	32.60	22.86	42.95	2.85
C18:0	8.72	3.19	17.12	1.43
C18u ³	22.35	15.29	41.14	2.57
CLA ⁴	0.40	0.02	1.25	0.11
TFA ⁵	1.64	0.75	7.74	0.41
SFA/UFA ⁶	2.75	1.08	4.16	0.36
Production trait				
305-d milk yield ⁷ (kg)	7,622.71	3,970.00	12,404.00	1,207.57
305-d protein yield ⁷ (kg)	262.44	149.00	404.00	40.22
305-d fat yield ⁷ (kg)	326.64	170.00	509.00	48.55
Protein concentration ⁸ (%)	3.50	2.67	4.59	0.30
Fat concentration ⁸ (%)	4.36	1.46	9.08	0.70

¹C5 to C15 includes the saturated fatty acids C5:0, C7:0, C9:0, C11:0, C13:0, and C15:0.

²C6 to C12 includes the saturated fatty acids C6:0, C8:0, C10:0, and C12:0.

³C18u includes the unsaturated C18 fatty acids C18:1 *trans*-6; C18:1 *trans*-9; C18:1 *trans*-10; C18:1 *trans*-11; C18:1 *cis*-9; C18:1 *cis*-11; C18:1 *cis*-12; C18:1 *cis*-13; C18:2 *cis*-9,12; C18:3 *cis*-9,12,15; C18:2 *cis*-9, *trans*-11; and C18:2 *trans*-10, *cis*-12.

⁴Conjugated linoleic acid (CLA) includes C18:2 *cis*-9, *trans*-11.

⁵Trans fatty acids (TFA) include C16:1 *trans*-9, C18:1 *trans*-6, C18:1 *trans*-9, C18:1 *trans*-10, and C18:1 *trans*-11.

⁶Saturated fatty acids (SFA) include C4:0, C5:0, C6:0, C7:0, C8:0, C9:0, C10:0, C11:0, C12:0, C13:0, C14:0 *iso*, C14:0, C15:0 *anteiso*, C15:0, C16:0 *iso*, C16:0, C17:0 *iso*, C17:0, C18:0, C20:0, C21:0, and C22:0. Unsaturated fatty acids (UFA) include C10:1; C12:1; C14:1 *cis*-9; C16:1 *trans*-9; C16:1 *cis*-9; C17:1 *cis*-9; C18:1 *trans*-6; C18:1 *trans*-9; C18:1 *trans*-10; C18:1 *trans*-11; C18:1 *cis*-9; C18:1 *cis*-11; C18:1 *cis*-12; C18:1 *cis*-13; C18:2 *cis*-9,12; C18:3 *cis*-9,12,15; C18:2 *cis*-9, *trans*-11; C18:2 *trans*-10, *cis*-12; C20:1 *cis*-11; C20:2 *cis*-11,14; C20:3 *cis*-8,11,14; and C22:1 *cis*-13.

⁷Provided by CRV (Arnhem, the Netherlands).

⁸Measured on single morning test-day milk samples collected between February and March 2005.

Statistical Analysis

Initial analyses were performed by using SAS 9.1 procedures (SAS Institute Inc., Cary, NC) to determine the significance of fixed effects. By using the MIXED procedure, linear mixed models were fitted as randomized block designs (Littell et al., 2006), with 394 herds as random blocks and individual cows as units of analysis.

Three modeling steps were performed. Model [1] was used to study the effects of milk fat composition on fertility. Model [1] included DIM (days between the date of first calving and the date of the milk sample), age at first calving, season of calving, and sire type (3 different sire groups: proven bulls, young bulls, or other proven bulls). Relationships between individuals were accounted for, and the total pedigree comprised 26,300 animals and was supplied by CRV. Variance components and fixed effect solutions were calculated by using the ASReml software package (Gilmour et al., 2006). Model [1] is written as

$$y_{ijklmno} = \mu + b_1 \text{DIM}_i + b_2 e^{-0.05 \text{DIM}} + b_3 \text{AFC}_j + b_4 \text{F}_k + \text{SEA}_l + \text{SCO}_m + \text{HERD}_n + a_o + e_{ijklmno}, \quad [1]$$

where y is the dependent variable (fertility trait), μ is the general mean, DIM is a fixed covariate accounting for the effect of DIM modeled with a Wilink curve (Wilink, 1987), AFC is a fixed covariate describing the effect of age at first calving, F is a fixed covariate accounting for the effect of one of the individual FA or FA groups, SEA is a fixed factor accounting for the effect of the calving season (June to August 2004, September to November 2004, or December 2004 to February 2005), SCO is a fixed factor capturing the effect of the differences in genetic level between proven bull daughters and young bull daughters, HERD is a random factor accounting for the effect of groups of animals sampled in the same herd, a is a random factor accounting for the additive genetic effect of the given animal, and e is the random residual effect. Each fertility trait was analyzed separately and each individual

Table 2. Descriptive statistics for fertility traits of 1,726 first-lactation Dutch Holstein-Friesian cows

Trait ¹	Cows (no.)	Mean	Minimum	Maximum	SD
CI	1,551	410.57	304	795	74.93
DFS	1,721	90.02	21	284	36.76
NINS	1,724	2.09	1	10	1.50
CR	1,726	0.90			
NR28	1,726	0.72			
NR56	1,726	0.60			
NR90	1,726	0.52			
CFI	1,726	0.44			

¹Fertility traits were interval between the first and second calving (CI), interval between calving and the first insemination (DFS), number of inseminations (NINS), calving rate (CR), nonreturn rate for insemination 28 d after the first service (NR28), nonreturn rate for insemination 56 d after the first service (NR56), nonreturn rate for insemination 90 d after the first service (NR90), and calving rate after the first insemination (CFI).

FA or FA group was also fitted separately (80 analyses in total).

To study the effects of the candidate genes, a model similar to model [1] was used. Model [2] excluded the covariate F and the correction for DIM (because no milk production trait measured on a single-day milk sample was involved), but included a fixed factor G that captured the effect of either the *DGAT1* genotype (AA, AK, or KK), or the *SCD1* genotype (AA, AV, or VV). Each fertility trait was analyzed separately and each polymorphism was also fitted separately (16 analyses in total). Model [2] is written as

$$y_{jlmnop} = \mu + b_1\text{AFC}_j + G_p + \text{SEA}_l + \text{SCO}_m + \text{HERD}_n + a_o + e_{jlmnop} \quad [2]$$

To study the effects of candidate genes adjusted for production traits, model [2] was extended with a fixed covariate P that corrected for the effect of 1 of the 5 milk production traits (model [3]). Each fertility trait was analyzed separately, each polymorphism was fitted separately, and the correction for each of the production traits was modeled separately (80 analyses in total). When correcting for protein or fat concentrations, model [3] also included DIM to correct for DIM (modeled with a Wilink curve) because those 2 production traits were measured on single test-day milk samples. Model [3] is written as

$$y_{jlmnopq} = \mu + b_1\text{AFC}_j + b_2P_q + G_p + \text{SEA}_l + \text{SCO}_m + \text{HERD}_n + a_o + e_{jlmnopq} \quad [3]$$

Because the models above assumed normally distributed residuals, we also fitted generalized linear mixed

models by using the GLIMMIX procedure in SAS 9.1 (Littell et al., 2006) for the count and binary variables. We assumed a Poisson distribution with a log link function for NINS and a binomial distribution with a logit link function for CR, NR28, NR56, NR90, and CFI. By fitting generalized linear models, the same fixed effects were significant as when the above linear models were fitted; therefore, we concluded that fitting linear mixed models was robust enough to handle the nonnormally distributed fertility traits.

In the above models, each individual FA, FA group, or polymorphism was fitted separately in a series of sequential analyses. In each analysis, the null hypothesis was of no FA or a polymorphism effect on the trait of interest. Because several fertility traits were analyzed, multiple hypothesis tests were performed. However, because of the correlations between those fertility traits, not all hypothesis tests were truly independent. To account for multiple testing, we considered the analyses of 1) CI, DFS, and NINS; 2) CR and CFI; and 3) NR28, NR56, and NR90 as 3 independent hypothesis tests. Bonferroni correction based on the method of Šidák (1967) was applied to account for multiple testing with regard to the 3 groups of fertility traits.

RESULTS AND DISCUSSION

Effects of Milk Fat Composition

To study the relationship between milk FA composition and reproductive performance, we used the FA profiles from 1,726 first-lactation Dutch Holstein-Friesian cows (model [1]). Estimated effects of the FA and their significance are presented in Table 3. The *trans* FA (TFA) were consistently found to affect reproduction, because this FA group had significant ($P < 0.05$) effects on several fertility traits. Greater TFA concentrations were associated with lower reproductive performance. More specifically, increased proportions of TFA within total fat resulted in larger (0.24) numbers of inseminations, lower (3.8%) calving rates, lower (5.5, 9.6, and 8.3%) nonreturn rates for insemination (28, 56, and 90 d after the first service, respectively), and lower (7.5%) calving rates after the first service. Associations between TFA concentration and NINS, NR56, NR90, and CFI were significant after Bonferroni correction. Although the relationships between TFA and CI and DFS were statistically nonsignificant, those results also suggest a possible negative effect of TFA for these traits.

In addition to the TFA, other significant ($P < 0.05$) relationships were found. The FA group C5 to C15 had a positive effect on reproduction because a greater proportion of C5 to C15 was associated with a higher (4.9%) calving rate. Larger concentrations of the individual FA

C18:0 were associated with lower (1.1%) calving rates, indicating a negative effect of C18:0. A negative effect was found for conjugated linoleic acid (CLA) C18:2 *cis*-9, *trans*-11 content, because a larger proportion of CLA was associated with lower (19.7%) nonreturn rates for insemination 90 d after the first service. However, because the effects for C5 to C15, C18:0, and CLA were found only on single fertility traits, and because the relationships became nonsignificant after Bonferroni correction, those findings are considered inconclusive. Regarding results for the remaining FA, no statistically significant association was observed between C4:0, C6 to C12, C14:0, C16:0, C18u, and the ratio of saturated FA to unsaturated FA and any of the fertility traits.

Milk FA composition can be changed by selective breeding (Soyeurt et al., 2007; Boebe et al., 2008; Stoop et al., 2008). Our findings regarding the effects of milk FA on fertility traits can be used to predict the correlated response of selection on FA composition. Whether a breeding program should aim to increase or decrease the concentration of a given FA or FA group to meet human health considerations should be weighed against correlated effects on the health and reproduction of the cow. Because of the contradictory published effects of TFA on human health (Bauman et al., 2006; German and Dillard, 2006; Jakobsen et al., 2006; Steijns, 2008), further research is needed to support the design of breeding programs. Our results indicate that selective breeding to decrease TFA concentrations could improve the fertility of cows.

Effects of the *DGAT1* K232A Polymorphism

To investigate the effect of the *DGAT1* polymorphism on reproductive performance, we studied a total of 1,726 animals (model [2]). The frequency of the K allele was 40%, and the distribution of the genotypes in the sample was in Hardy-Weinberg equilibrium. The estimated effects of *DGAT1* variants are presented in Table 4. Our results indicated that the *DGAT1* polymorphism had a significant ($P < 0.05$) effect on 2 fertility traits: NR28 and NR56. Results for the different *DGAT1* variants showed a nonadditive pattern. More specifically, the AK genotype was associated with the lowest estimates for both NR28 and NR56, followed by the AA and the KK genotypes. Relative to cows with the AA genotype, cows with the AK genotype are expected to have lower (6 and 4%) nonreturn rates at 28 and 56 d after the first service. Cows with the KK genotype, however, are expected to have higher (1 and 5%) nonreturn rates at 28 and 56 d, respectively, when compared with cows with the AA genotype. Nevertheless, the effect of *DGAT1* on NR28 and NR56 became nonsignificant after Bonferroni correction, meaning the

Table 3. Effects of individual fatty acids or fatty acid groups¹ on fertility traits² of 1,726 first-lactation Dutch Holstein-Friesian cows³

Fatty acid	CI (n = 1,551)	DFS (n = 1,721)	NINS (n = 1,724)	CR (n = 1,726)	NR28 (n = 1,726)	NR56 (n = 1,726)	NR90 (n = 1,726)	CFI (n = 1,726)
C4:0	-8.013 ± 7.102	-0.382 ± 3.263	-0.209 ± 0.130	-0.042 ± 0.026	0.011 ± 0.040	0.037 ± 0.044	0.059 ± 0.045	0.037 ± 0.044
C5 to C15	5.804 ± 6.211	1.361 ± 2.920	0.049 ± 0.114	0.049 ± 0.023*	-0.013 ± 0.035	-0.041 ± 0.039	-0.053 ± 0.040	-0.032 ± 0.039
C6 to C12	-1.493 ± 1.611	0.042 ± 0.757	-0.009 ± 0.030	0.002 ± 0.006	-0.005 ± 0.009	-0.009 ± 0.010	-0.001 ± 0.010	-0.001 ± 0.010
C14:0	-2.211 ± 2.123	-0.218 ± 0.988	0.013 ± 0.039	0.003 ± 0.008	-0.012 ± 0.012	-0.021 ± 0.013	-0.010 ± 0.013	-0.004 ± 0.013
C16:0	0.303 ± 0.703	-0.113 ± 0.328	-0.009 ± 0.013	0.004 ± 0.003	0.000 ± 0.004	0.005 ± 0.004	0.003 ± 0.004	0.004 ± 0.004
C18:0	-1.795 ± 1.418	-1.007 ± 0.640	0.005 ± 0.025	-0.011 ± 0.005*	0.000 ± 0.008	-0.001 ± 0.009	0.006 ± 0.009	0.003 ± 0.009
C18u	0.916 ± 0.791	0.412 ± 0.372	0.014 ± 0.014	-0.003 ± 0.003	0.003 ± 0.004	0.000 ± 0.005	-0.003 ± 0.005	-0.004 ± 0.005
CLA	9.090 ± 18.18	8.531 ± 8.656	0.495 ± 0.325	-0.009 ± 0.065	-0.125 ± 0.099	-0.178 ± 0.111	-0.197 ± 0.113*	-0.149 ± 0.111
TFA	5.037 ± 5.148	2.030 ± 2.485	0.240 ± 0.089**	-0.038 ± 0.018*	-0.055 ± 0.027*	-0.096 ± 0.030**	-0.083 ± 0.031**	-0.075 ± 0.030**
SFA/UFA	-7.706 ± 5.530	-4.150 ± 2.567	-0.072 ± 0.100	0.005 ± 0.020	-0.011 ± 0.031	0.000 ± 0.034	0.019 ± 0.035	0.022 ± 0.034

¹Groups of fatty acids are as follows: C5 to C15 includes the saturated fatty acids C5:0, C7:0, C9:0, C11:0, C13:0, and C15:0. C6 to C12 includes the saturated fatty acids C6:0, C8:0, C10:0, and C12:0. C18u includes the unsaturated C18 fatty acids C18:1 *trans*-6; C18:1 *trans*-9; C18:1 *trans*-10; C18:1 *trans*-11; C18:1 *cis*-9; C18:1 *cis*-11; C18:1 *cis*-12; C18:1 *cis*-13; C18:2 *cis*-9,12; C18:3 *cis*-9,12,15; C18:2 *cis*-9, *trans*-11; and C18:2 *trans*-10, *cis*-12. Conjugated linoleic acid (CLA) includes C18:2 *cis*-9, *trans*-11. *Trans* fatty acids (TFA) include C16:1 *trans*-9, C18:1 *trans*-6, C18:1 *trans*-9, C18:1 *trans*-10, and C18:1 *trans*-11. Saturated fatty acids (SFA) include C4:0, C5:0, C6:0, C7:0, C8:0, C9:0, C10:0, C11:0, C12:0, C13:0, C14:0 *iso*, C14:0, C15:0 *anteiso*, C15:0, C16:0 *iso*, C16:0, C17:0 *iso*, C17:0, C18:0, C20:0, C21:0, and C22:0. Unsaturated fatty acids (UFA) include C10:1; C12:1; C14:1 *cis*-9; C16:1 *trans*-9; C16:1 *cis*-9; C17:1 *cis*-9; C18:1 *trans*-6; C18:1 *trans*-9; C18:1 *trans*-10; C18:1 *trans*-11; C18:1 *cis*-9; C18:1 *cis*-11; C18:1 *cis*-12; C18:1 *cis*-13; C18:2 *cis*-9,12; C18:3 *cis*-9,12,15; C18:2 *cis*-9, *trans*-11; C18:2 *trans*-10, *cis*-12; C20:1 *cis*-11; C20:2 *cis*-11,14; C20:3 *cis*-8,11,14; and C22:1 *cis*-13.

²Fertility traits were interval between the first and second calving (CI), interval between calving and the first insemination (DFS), number of inseminations (NINS), calving rate (CR), nonreturn rate for insemination 28 d after the first service (NR28), nonreturn rate for insemination 56 d after the first service (NR56), nonreturn rate for insemination 90 d after the first service (NR90), and calving rate after the first insemination (CFI).

³Values after ± signs are corresponding SE.

*Significant ($P < 0.05$); **significant after Bonferroni correction.

Table 4. Effect of *DGAT1* polymorphism on fertility traits¹ of 1,726 first-lactation Dutch Holstein-Friesian cows²

Trait	Cows (no.)	Predicted mean of AA genotype	AA-AK	AA-KK	P-value
CI	1,551	398.21	4.65 ± 4.22	-0.61 ± 5.82	0.439
DFS	1,721	88.67	0.34 ± 1.93	-3.97 ± 2.69	0.212
NINS	1,724	1.79	0.06 ± 0.08	-0.05 ± 0.11	0.499
CR	1,726	0.89	0.03 ± 0.02	0.02 ± 0.02	0.140
NR28	1,726	0.79	-0.06 ± 0.02	0.01 ± 0.03	0.024*
NR56	1,726	0.68	-0.04 ± 0.03	0.05 ± 0.04	0.037*
NR90	1,726	0.60	-0.02 ± 0.03	0.04 ± 0.04	0.275
CFI	1,726	0.53	-0.02 ± 0.03	0.04 ± 0.04	0.238

¹Fertility traits were interval between the first and second calving (CI), interval between calving and the first insemination (DFS), number of inseminations (NINS), calving rate (CR), nonreturn rate for insemination 28 d after the first service (NR28), nonreturn rate for insemination 56 d after the first service (NR56), nonreturn rate for insemination 90 d after the first service (NR90), and calving rate after the first insemination (CFI).

²Values after ± signs are corresponding SE.

*Significant ($P < 0.05$); nonsignificant after Bonferroni correction.

results are suggestive rather than conclusive. Associations between *DGAT1* and the other fertility traits (CI, DFS, NINS, CR, NR90, and CFI) were statistically nonsignificant.

Because the *DGAT1* polymorphism has a strong influence on milk fat content and other economically important milk production traits (Grisart et al., 2002; Winter et al., 2002), the effect of *DGAT1* on fertility might be due to its correlated effect on the production traits. Further analyses, therefore, were performed by correcting for 1 of the 5 production traits (model [3]). Table 5 shows the estimated effects of the *DGAT1* variants after correcting for production traits. Similar to the results above, the *DGAT1* polymorphism had significant ($P < 0.05$) effects on NR28 and NR56, independent of which production trait was included in the model, although the results remained significant post Bonferroni correction only for NR28 after adjusting for fat concentrations. Moreover, the pattern of the estimated effects of different *DGAT1* variants remained nonadditive (heterozygous disadvantage). Among the 3 genotypes, the AK variant was consistently associated with the lowest nonreturn rates at 28 and 56 d. In short, the results of model [3] showed that the effect of *DGAT1* on milk production traits could not explain the association found between *DGAT1* and fertility traits. Furthermore, the pattern of heterozygous animals being associated with lower nonreturn rates than homozygous animals remained. In conclusion, our results from model [2] and model [3] suggest a potential effect of *DGAT1* on nonreturn rates for insemination 28 and 56 d after the first insemination.

Research has suggested that *DGAT1* might play a role in reproduction. First, in a recent study Schmidt et al. (2006) found that *DGAT1* was expressed in ovaries of hormone-treated rats. According to Schmidt et

al. (2006), this finding suggests that *DGAT1* might be important for the cellular proliferation and differentiation needed for reorganization in the ovary, because the gene is important for mitosis in other tissues and is induced by FSH. Second, bovine chromosome 14, where *DGAT1* is located, has been studied extensively for QTL related to many economically important traits in cattle. Several studies have reported QTL, including *DGAT1*, on bovine chromosome 14 that affect various (male) fertility traits in different dairy cattle breeds (Daetwyler et al., 2008; Kolbehdari et al., 2008). In short, there is evidence that *DGAT1* could affect reproductive performance.

However, little is known about a direct association between the *DGAT1* polymorphism and reproductive traits in cattle. Kaupe et al. (2007) studied the allele-substitution effect on reproductive traits and showed a negative effect of the K allele on maternal nonreturn rate. Oikonomou et al. (2009), in contrast, found the A allele to be associated with a greater number of inseminations, lower conception rates, and a greater chance of reproductive problems. Kaupe et al. (2007) and Oikonomou et al. (2009) studied the gene substitution effect, meaning that the effects of the *DGAT1* polymorphism were captured by linear regression coefficients estimated for the covariate corresponding to the number of copies of the substitution allele. The pattern of our results, however, was different from those 2 studies.

Investigating the effects of the *DGAT1* polymorphism on fertility traits is important because this information can be used to evaluate selective breeding programs that exploit this polymorphism. As proposed by Schennink et al. (2007), the *DGAT1* polymorphism can be used in selective breeding to change the milk FA composition. They demonstrated that increasing the frequency of the A allele would be desirable from a public health

Table 5. Effect of *DGAT1* polymorphism on fertility traits¹ when correcting for production traits of 1,726 first-lactation Dutch Holstein-Friesian cows²

Trait	Cows (no.)	Predicted mean of AA genotype	AA-AK	AA-KK	P-value
Correcting for 305-d milk yield					
CI	1,551	396.06	8.18 ± 4.17	8.54 ± 5.84	0.122
DFS	1,721	88.25	1.80 ± 1.92	-0.40 ± 2.73	0.523
NINS	1,724	1.79	0.11 ± 0.08	0.06 ± 0.11	0.393
CR	1,726	0.89	0.03 ± 0.02	0.03 ± 0.02	0.110
NR28	1,726	0.79	-0.06 ± 0.02	-0.01 ± 0.03	0.021*
NR56	1,726	0.68	-0.04 ± 0.03	0.03 ± 0.04	0.051*
NR90	1,726	0.60	-0.03 ± 0.03	0.01 ± 0.04	0.360
CFI	1,726	0.53	-0.03 ± 0.03	0.02 ± 0.04	0.328
Correcting for 305-d protein yield					
CI	1,551	397.37	5.36 ± 4.18	3.26 ± 5.80	0.446
DFS	1,721	88.72	0.72 ± 1.92	-2.52 ± 2.69	0.432
NINS	1,724	1.80	0.08 ± 0.08	0.01 ± 0.11	0.573
CR	1,726	0.89	0.03 ± 0.02	0.03 ± 0.02	0.114
NR28	1,726	0.79	-0.06 ± 0.02	0.00 ± 0.03	0.025*
NR56	1,726	0.68	-0.04 ± 0.03	0.04 ± 0.04	0.051*
NR90	1,726	0.60	-0.02 ± 0.03	0.02 ± 0.04	0.360
CFI	1,726	0.53	-0.02 ± 0.03	0.03 ± 0.04	0.304
Correcting for 305-d fat yield					
CI	1,551	401.46	-0.62 ± 4.31	-7.32 ± 5.92	0.413
DFS	1,721	90.06	-1.31 ± 1.97	-6.17 ± 2.74	0.072
NINS	1,724	1.84	0.01 ± 0.08	-0.12 ± 0.11	0.459
CR	1,726	0.90	0.03 ± 0.02	0.02 ± 0.02	0.241
NR28	1,726	0.78	-0.05 ± 0.03	0.01 ± 0.03	0.031*
NR56	1,726	0.67	-0.03 ± 0.03	0.06 ± 0.04	0.038*
NR90	1,726	0.59	-0.01 ± 0.03	0.04 ± 0.04	0.266
CFI	1,726	0.53	-0.01 ± 0.03	0.05 ± 0.04	0.230
Correcting for protein concentration					
CI	1,551	400.32	7.59 ± 4.33	4.00 ± 6.07	0.212
DFS	1,721	90.89	1.91 ± 1.99	-1.18 ± 2.82	0.375
NINS	1,724	1.87	0.07 ± 0.08	-0.04 ± 0.12	0.497
CR	1,726	0.90	0.03 ± 0.02	0.01 ± 0.02	0.300
NR28	1,726	0.78	-0.06 ± 0.03	0.01 ± 0.04	0.026*
NR56	1,726	0.67	-0.03 ± 0.03	0.06 ± 0.04	0.039*
NR90	1,726	0.57	-0.02 ± 0.03	0.04 ± 0.04	0.261
CFI	1,726	0.53	-0.02 ± 0.03	0.04 ± 0.04	0.272
Correcting for fat concentration					
CI	1,551	397.75	12.29 ± 4.52	12.95 ± 6.58	0.023*
DFS	1,721	90.06	2.96 ± 2.08	0.95 ± 3.05	0.307
NINS	1,724	1.81	0.17 ± 0.08	0.14 ± 0.12	0.148
CR	1,726	0.90	0.03 ± 0.02	0.02 ± 0.03	0.191
NR28	1,726	0.80	-0.08 ± 0.03	-0.03 ± 0.04	0.009**
NR56	1,726	0.70	-0.07 ± 0.03	-0.01 ± 0.04	0.032*
NR90	1,726	0.60	-0.06 ± 0.03	-0.04 ± 0.04	0.135
CFI	1,726	0.56	-0.06 ± 0.03	-0.03 ± 0.04	0.128

¹Fertility traits were interval between the first and second calving (CI), interval between calving and the first insemination (DFS), number of inseminations (NINS), calving rate (CR), nonreturn rate for insemination 28 d after the first service (NR28), nonreturn rate for insemination 56 d after the first service (NR56), nonreturn rate for insemination 90 d after the first service (NR90), and calving rate after the first insemination (CFI).

²Values after ± signs are corresponding SE.

*Significant ($P < 0.05$); **significant after Bonferroni correction.

point of view because of the association of this variant with more unsaturated FA. According to Schennink et al. (2007), an increase in the frequency of the A allele could lead to a decrease in the C16:0 fraction and an increase in the unsaturated C18 fraction by approximately 5 to 10%. Our results suggest that such a breeding goal could have consequences on reproduction.

Replacing cows with the AK genotype with cows with the AA genotype could be positive, because it could lead to improved nonreturn rates for insemination (28 and 56 d) after the first service. However, increasing the frequency of the A allele by replacing cows with the KK genotype could have undesirable effect on reproductive performance. Because the allele frequencies found in

Table 6. Effect of *SCD1* polymorphism on fertility traits¹ of 1,661 first-lactation Dutch Holstein-Friesian cows²

Trait	Cows (no.)	Predicted mean of AA genotype	AA–AV	AA–VV	P-value
CI	1,493	398.21	2.75 ± 4.05	5.72 ± 7.81	0.676
DFS	1,656	87.22	0.41 ± 1.88	1.96 ± 3.67	0.866
NINS	1,659	1.84	–0.03 ± 0.08	–0.04 ± 0.15	0.928
CR	1,661	0.90	0.02 ± 0.02	0.04 ± 0.03	0.318
NR28	1,661	0.76	0.01 ± 0.02	–0.01 ± 0.05	0.894
NR56	1,661	0.67	–0.01 ± 0.03	–0.01 ± 0.05	0.923
NR90	1,661	0.59	0.00 ± 0.03	–0.02 ± 0.05	0.935
CFI	1,661	0.53	–0.02 ± 0.03	–0.02 ± 0.05	0.814

¹Fertility traits were interval between the first and second calving (CI), interval between calving and the first insemination (DFS), number of inseminations (NINS), calving rate (CR), nonreturn rate for insemination 28 d after the first service (NR28), nonreturn rate for insemination 56 d after the first service (NR56), nonreturn rate for insemination 90 d after the first service (NR90), and calving rate after the first insemination (CFI).

²Values after ± signs are corresponding SE.

our sample suggest a considerably greater proportion of AK than KK animals, selecting for cows with the AA genotype seems to be the most desirable with regard to nutritional quality concerns and the reproductive performance of the cows.

Effects of the *SCD1* A293V Polymorphism

To study the effects of the *SCD1* polymorphism on fertility, we analyzed a sample of 1,661 first-lactation Dutch Holstein-Friesian cows (model [2]). The frequency of the A allele was approximately 73%, and the genotypes were in Hardy-Weinberg equilibrium. The estimated effects of the *SCD1* variants are presented in Table 6. As the results indicate, we found no significant effect on any of the reproductive traits. This finding was further reinforced by the results of the extended model correcting for production traits (model [3]; results not shown).

Stearoyl-CoA desaturase (**SCD**), which is also called Δ^9 -desaturase, is an enzyme that introduces a *cis* double bond between carbon atoms C9 and C10 of the FA chain, thereby desaturating saturated FA to mono-unsaturated FA. Several SCD genes have been isolated in several species, and the isoforms are differentially distributed in different tissues.

Recent research has suggested that SCD isoforms might play a role in female fertility in mammalian species. Moreau et al. (2006) were the first to show the expression of SCD1 and SCD2 in the rat ovary. They found several-fold higher expression for SCD2 than for SCD1, indicating that SCD2 is responsible for Δ^9 -desaturase activity in the ovary. Because the oocyte is rich in unsaturated FA, according to Moreau et al. (2006), monounsaturated FA formed in the cumulus cells via SCD2 activation can play a vital role in fertility. This assertion is based on the fact that removal of

cumulus cells at the time of fertilization often results in lower fertilization rates in most mammalian species (Wang and Niwa, 1995; Zhang et al., 1995).

In the literature we found only one genome-wide scan that reported a QTL on bovine chromosome 26 (where *SCD1* is located) affecting female reproductive performance. Thomasen et al. (2008) detected a QTL on bovine chromosome 26 that affects calving traits in Danish Holstein cattle.

To our knowledge, however, no previous study investigating a direct association between any isoforms of the SCD gene and reproductive traits in any mammalian species has been published. The present study did not detect any significant association between the Δ^9 -desaturase isoform SCD1 and female reproductive performance in dairy cattle. Because of its effect on milk FA composition, *SCD1* has been considered as a candidate gene to be used in breeding programs for improving the nutritional value of milk (Mele et al., 2007; Moioli et al., 2007; Schennink et al., 2008). Our findings indicate that selection based on *SCD1* genotypes would not affect the reproductive performance of the cows.

CONCLUSIONS

The objective of this study was to examine the effect of milk fat composition, *DGAT1*, and *SCD1* on fertility traits in commercial Dutch Holstein-Friesian dairy cattle. Data on 1,745 first-lactation cows were analyzed by fitting linear mixed models. Greater concentrations of TFA within total milk fat negatively affected reproductive performance. Our results suggest that *DGAT1* has a potential effect on nonreturn rates for insemination 28 and 56 d after the first service. Results for *DGAT1* display a nonadditive pattern, with heterozygous genotypes associated with lower estimates

for nonreturn rates than homozygous genotypes. Our findings suggest that a breeding program selecting for animals with the AA genotype would be the most desirable with regard to the nutritional quality of milk and the reproductive performance of cows. Results of this study can be used to assess the (economic) effects of changing milk fat composition. Consequently, selective breeding programs can be better evaluated before implementation.

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