Genome-wide scan for bovine milk-fat composition. I. Quantitative trait loci for short- and medium-chain fatty acids

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ABSTRACT

A genome-wide scan was performed to identify quantitative trait loci (QTL) for short- and medium-chain fatty acids (expressed in wt/wt %). Milk samples were available from 1,905 cows from 398 commercial herds in the Netherlands, and milk-fat composition was measured by gas chromatography. DNA was available from 7 of the paternal half-sib families: 849 cows and their 7 sires. A genetic map was constructed comprising 1,341 SNP and 2,829 cM, with an average information content of 0.83. Multimarker interval mapping was used in an across-family regression on corrected phenotypes for the 7 half-sib families. Four QTL were found: on *Bos taurus* autosome (BTA) 6, a QTL was identified for C6:0 and C8:0; on BTA14, a QTL was identified for fat percentage, all odd-chain fatty acids, and C14:0, C16:0, C16:1, and their unsaturation indices; on BTA19, a QTL affected C14:0; and on BTA26, a QTL was identified for the monounsaturated fatty acids and their unsaturation indices. The QTL explained 3 to 19% of phenotypic variance. Furthermore, 49 traits with suggestive evidence for linkage were found on 21 chromosomes. Additional analyses revealed that the QTL on BTA14 was most likely caused by a mutation in *DGAT1*, whereas the QTL on BTA26 was most likely caused by a mutation in the *SCD1* gene. Quantitative trait loci that affect specific fatty acids might increase the understanding of physiological processes regarding fat synthesis and the position of the causal genes.

Key words: genome-wide scan, quantitative trait locus, fatty acid, dairy cattle

INTRODUCTION

Changing milk fatty acid proportions to a milk-fat composition that contributes to improved human

health has received considerable attention in the last years (Jensen, 2002; German and Dillard, 2006; Soyeurt et al., 2006, 2007; Vlaeminck et al., 2006; Stoop et al., 2008).

Short- and medium-chain C4 to C17 saturated and monounsaturated milk fatty acids (FA) are largely synthesized de novo in the mammary gland. Saturated even-chain C4:0 to C16:0 FA are synthesized from C2 and C4 precursors. For C16:0, about 50% comes from de novo synthesis in the mammary gland and 50% from blood. Odd-chain FA are mainly derived from rumen bacteria and partly from de novo synthesis from C3 precursors. Monounsaturated FA are suggested to originate from Δ^9 -desaturase enzyme activity (MacGibbon and Taylor, 2006). Several studies showed genetic variation in milk FA proportions (Soyeurt et al., 2007; Bobe et al., 2008; Stoop et al., 2008). Stoop et al. (2008) showed substantial genetic variation in FA, with intra-herd heritabilities ranging from 0.22 to 0.71. Identifying the genes responsible for this genetic variation is expected to contribute greatly to our understanding of milk FA synthesis and to enhance opportunities to improve milk-fat composition through selective breeding.

Several studies have looked at the effects of genes that are expected to play a role in milk-fat synthesis (Clarke, 1993; Falaki et al., 1997; Stephens et al., 1999; Brym et al., 2004; Cohen et al., 2004; Schnabel et al., 2005; Roy et al., 2006b; Viitala et al., 2006; Khatib et al., 2007; Morris et al., 2007). Although many of these studies found an effect on fat percentage or fat yield, results on milk-fat composition are very limited. Recent studies found significant effects of the diacylglycerol acyltransferase 1 (*DGAT1*) K232A mutation (Schennink et al., 2007) and of the stearoyl-coenzyme A desaturase 1 (*SCD1*) A293V mutation (Schennink et al., 2008). However, these 2 genes explain only part of the genetic variation in milk-fat composition, and it is expected that more genes are involved.

Our aim was to identify QTL for fatty acid composition of bovine milk by performing a genome-wide scan. This paper presents results for short- and medium-chain FA. The accompanying paper by Schennink et al. (2009) presents results for long-chain FA.

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MATERIALS AND METHODS

Phenotypes

This study was part of the Dutch Milk Genomics Initiative, which focuses on genetic aspects of milk composition. To study milk-fat composition, milk samples were available from 1,905 cows from 398 commercial herds in the Netherlands. Cows descended from 1 of 5 proven bulls ($n = 871$), 1 of 50 young bulls ($n = 844$), or 1 of 46 other proven bulls ($n = 190$). The latter group ensured 3 sampled cows per farm. Each cow was more than 87.5% Holstein-Friesian, was between d 63 and 282 of first lactation, and was milked twice a day. One morning milk sample of 500 mL per cow was collected in the winter of 2005. Sample bottles contained sodium azide (0.03 wt/wt%) for conservation. Milk-fat composition was measured by gas chromatography at the laboratory of the Netherlands Controlling Authority for Milk and Milk Products (Leusden, the Netherlands) as described by Schennink et al. (2007) and Stoop et al. (2008). The FA were measured as weight proportion of total fat weight. In this study, data are presented for 24 traits related to short- and medium-chain FA: overall fat percentage; saturated C4 to C17 FA; monounsaturated C10:1, C12:1, C14:1, C16:1, and C17:1; and the unsaturation indices of these FA. The unsaturation index is an indication of the activity of the Δ^9 -desaturase enzyme and was calculated as

$$\frac{\text{cis-9 FA}}{\text{cis-9 FA} + \text{saturated FA}} \times 100,$$

(Kelsey et al., 2003); for example,

$$\frac{\text{C14:1}}{\text{C14:1} + \text{C14:0}} \times 100.$$

Percentage of fat was determined from a 10-mL milk subsample by infrared spectroscopy by using a Fourier-transform interferogram (MilkoScan FT 6000, Foss Electric, Hillerød, Denmark) at the certified laboratory of the Milk Control Station (Zutphen, the Netherlands). Intraherd heritabilities were calculated as described by Stoop et al. (2008), based on the 1,905 animals from the current study, instead of the 1,933 animals from Stoop et al. (2008). The 28 missing animals had genotypes that conflicted with milk protein types.

Genotypes, Markers, and Linkage Map

Blood and semen samples were collected for DNA isolation. For the genome scan, genotypes were available from 7 paternal half-sib families of in total 849 cows

and 7 sires. The 7 sires were the 5 proven bulls and 2 of the young bulls from which daughter phenotypes were collected. The 7 half-sib families consisted of 193, 179, 170, 166, 91, 29, and 21 cows, respectively. A total of 1,536 SNP were selected either based on heterozygosity in the sires, as known from previous experiments in the Dutch Holstein-Friesian population by CRV (Arnhem, the Netherlands) or from dbSNP, the National Center for Biotechnology Information SNP database (<http://www.ncbi.nlm.nih.gov/projects/SNP>). Genotyping was performed by the GoldenGate assay (Illumina, San Diego, CA). Additionally, genotypes for *SCD1* and *DGAT1* were available from previous studies. Genotypes for *SCD1* were obtained by a SNaPshot assay (Schennink et al., 2008) and genotypes for *DGAT1* were obtained by an allelic discrimination method (Schennink et al., 2007). Genotypes of *DGAT1* and *SCD1* were available for 1,687 out of 1,905 cows.

Of all SNP, 1,341 SNP were successfully incorporated into the linkage map of 2,829 cM that was calculated with CRI-MAP (Green et al., 1990). Full details on the genotypings, SNP, and construction of the linkage map are reported by Schopen et al. (2009). When markers mapped at the same position, they were placed 0.1 cM apart to ensure that all markers were included in the analysis by the QTL program. The resulting map of 2,831 cM was used in the QTL analysis.

QTL Mapping

Phenotypes were precorrected for the systematic effects of DIM, age at first calving, season of calving, and herd. The effects were estimated using an Animal Model in ASReml (Gilmour et al., 2002) on all 1,905 cows with phenotypes, as described by Schennink et al. (2007) and Stoop et al. (2008). Multimarker interval mapping was used in an across-family regression on the corrected phenotypes for the 7 half-sib families that were genotyped (Knott et al., 1996). The regression was performed for each trait and each chromosome by using the following regression model (Spelman et al., 1996):

$$Y_{ijk} = \mu + s_i + b_{ik}X_{ijk} + e_{ijk}, \quad [1]$$

where Y_{ijk} is the corrected phenotypic observation for daughter j nested within sire i at position k , μ is the overall mean, s_i is the fixed effect of sire i , b_{ik} is the regression coefficient for sire i at position k , X_{ijk} is the probability of daughter j inheriting gamete 1 from sire i at position k , and e_{ijk} is the random residual effect for daughter j nested within sire i at position k .

Significance thresholds were calculated by permutation testing (Churchill and Doerge, 1994). Chromosome-wise significance levels ($P_{\text{chromosome}}$) were obtained

Table 1. Mean proportion, phenotypic SD (σ_P), and intraherd heritability (h^2) and standard error (subscript) of short- and medium-chain fatty acids (FA) and unsaturation indices, measured on 1,905 cows¹

Trait	Mean (wt/wt%)	σ_P	h^2_{SE}
C4:0	3.50	0.24	0.44 _{0.09}
C5:0	0.03	0.01	0.14 _{0.05}
C6:0	2.22	0.14	0.47 _{0.10}
C7:0	0.03	0.01	0.17 _{0.07}
C8:0	1.37	0.12	0.61 _{0.11}
C9:0	0.04	0.02	0.25 _{0.08}
C10:0	3.03	0.35	0.72 _{0.12}
C11:0	0.08	0.03	0.34 _{0.09}
C12:0	4.11	0.46	0.64 _{0.11}
C13:0	0.11	0.04	0.19 _{0.07}
C14:0	11.61	0.78	0.62 _{0.11}
C15:0	1.17	0.16	0.30 _{0.08}
C16:0	32.59	2.15	0.43 _{0.11}
C17:0	0.45	0.04	0.33 _{0.09}
C10:1	0.37	0.06	0.34 _{0.09}
C12:1	0.12	0.02	0.38 _{0.09}
C14:1	1.36	0.23	0.34 _{0.08}
C16:1	1.44	0.30	0.44 _{0.09}
C17:1	0.18	0.03	0.43 _{0.10}
C10 index ²	10.89	1.76	0.37 _{0.09}
C12 index	2.74	0.49	0.36 _{0.09}
C14 index	10.51	1.68	0.45 _{0.09}
C16 index	4.24	0.76	0.47 _{0.09}
C17 index	28.29	2.58	0.47 _{0.10}
Fat %	4.36	0.64	0.49 _{0.10}

¹Individual FA in this table sum to 63.8 wt/wt%. Other FA include branched-chain FA (no results shown) and long-chain FA (see Schennink et al., 2009).

²The unsaturation index was calculated as $\frac{cis-9\text{ FA}}{cis-9\text{ FA} + \text{saturated FA}} \times 100$.

by 10,000 permutations. Genome-wise significance levels (P_{genome}) were derived from the chromosome-wise significance levels using a Bonferroni correction (De Koning et al., 1998): $P_{\text{genome}} = 1 - (1 - P_{\text{chromosome}})^{1/r}$, where r is the ratio of the chromosome length over the genome length. Significant linkage was declared at the 5% genome-wise significance threshold, and suggestive linkage was declared at the 5% chromosome-wise significance threshold (Lander and Kruglyak, 1995). The confidence interval for the most likely QTL position was based on 1,000 bootstraps (Visscher et al., 1996). Allele substitution effects were estimated for the significant QTL within each of the 7 families at the most likely QTL position from model [1] using SAS, version 9.1 (SAS Institute, 2004).

Additional analyses were done to investigate whether the effects of polymorphisms in the *DGAT1* and *SCD1* genes could explain the QTL detected in this study. For this analysis, phenotypes were additionally corrected for the *DGAT1* K232A as well as the *SCD1* A293V

genotypes by including these as fixed effects in the Animal Model. Subsequently, the QTL regression was rerun for all adjusted trait values.

RESULTS

Table 1 shows the average weight proportion (wt/wt%) and intraherd heritability of the included FA and indices.

QTL

Quantitative trait loci for short- and medium-chain FA were identified on 21 chromosomes in this study and are summarized in Table 2. All QTL that exceeded the threshold for suggestive linkage ($P_{\text{chromosome}} < 0.05$) are reported. Significant statistical evidence ($P_{\text{genome}} < 0.05$) for QTL was found for 23 traits on 4 distinct chromosomal regions on *Bos taurus* autosome (BTA) 6, BTA14, BTA19, and BTA26. Figures 1 to 4 show the graphs with test statistic distributions for these 4 chromosomes; for BTA14 and BTA26, only a selection of the significant traits is shown (see Table 2 for all traits). Allele substitution effects for significant QTL are shown in Table 3. Results are reported and discussed per chromosome.

On BTA6, a significant QTL near 55 cM affected C6:0 and C8:0 (Table 2, Figure 1). The QTL on BTA6 significantly segregated within families 1, 5, and 6, with an effect of about 0.5 phenotypic SD (Table 3). In family 1, the difference between the 2 daughter groups was 0.08 (wt/wt%) for C6:0, and 0.07 (wt/wt%) for C8:0. In family 5, the difference for C6:0 was 0.05 (wt/wt%), but was not significant, whereas the difference for C8:0 was also 0.07 (wt/wt%). In family 6, the difference for C6:0 was 0.10 (wt/wt%). The QTL explained approximately 3% of the phenotypic variation for both C6:0 and C8:0.

On BTA14, a significant QTL at the centromeric end of the chromosome affected all odd-chain FA and C14:0, C16:0, and C16:1, with their unsaturation indices (Table 2, Figure 2). The allele that positively affected C14:0 had a negative effect on the other traits (Table 3). The size of the QTL effect was generally 0.5 to 1 phenotypic SD (Table 3). Sires of families 1, 2, 3, 4, and 7 were heterozygous for the QTL. The QTL explained between 3 and 19% of phenotypic variance for the different traits. This QTL also affected several long-chain FA and the total ratio of saturated and unsaturated FA (Schennink et al., 2009). This QTL mapped to the approximate location of the *DGAT1* gene.

On BTA19, a significant QTL was found for C14:0 (60 cM; Table 2, Figure 3). The QTL on BTA19 segregated significantly within families 2, 4, and 7, with an

Table 2. Location and characteristics of suggestive and significant QTL affecting short- and medium-chain fatty acids, unsaturation indices, and fat percentage

<i>Bos taurus</i> autosome	Trait	<i>F</i> -statistic	Position (cM)	95% Confidence interval (cM)	<i>P</i> _{chromosome}	<i>P</i> _{genome}	<i>DGAT1</i> and <i>SCD1</i> corrected ¹		
							Position (cM)	<i>P</i> _{chromosome}	<i>P</i> _{genome}
1	C16:0	3.32	64	25 to 112	0.034	0.492	—	—	—
1	C16:1	—	—	—	—	—	71	0.048	0.621
1	C17:0	3.84	77	28 to 145	0.010	0.185	132	0.039	0.544
2	C4:0	3.74	112	0 to 129	0.010	0.195	112	0.017	0.314
2	C6:0	3.80	110	14 to 127	0.006	0.120	110	0.014	0.270
2	C15:0	3.17	53	0 to 78	0.043	0.620	—	—	—
3	C17:1	2.94	94	0 to 126	0.045	0.646	—	—	—
4	C7:0	—	—	—	—	—	85	0.009	0.194
4	C13:0	—	—	—	—	—	86	0.047	0.665
4	C16:0	—	—	—	—	—	64	0.022	0.405
4	C17 index	3.79	31	0 to 79	0.006	0.125	—	—	—
5	C10 index	4.81	95	49 to 114	0.003	0.071	96	0.010	0.208
5	C12 index	3.19	94	44 to 114	0.033	0.538	—	—	—
5	C14 index	3.17	82	43 to 114	0.040	0.609	—	—	—
6	C6:0	4.11	53	16 to 71	0.002	0.050*	53	0.005	0.105
6	C8:0	4.42	57	19 to 72	0.002	0.034*	57	0.003	0.067
6	C10:0	—	—	—	—	—	57	0.050	0.694
6	C16:1	—	—	—	—	—	57	0.036	0.574
6	C17:1	3.15	57	8 to 72	0.025	0.446	—	—	—
6	C17 index	2.96	57	2 to 107	0.042	0.627	—	—	—
6	Fat %	3.07	81	3 to 109	0.033	0.538	—	—	—
7	C4:0	3.51	0	0 to 126	0.014	0.276	0	0.022	0.390
8	C15:0	3.10	67	23 to 105	0.048	0.697	65	0.029	0.504
8	Fat %	3.23	82	45 to 113	0.022	0.410	—	—	—
9	C5:0	2.95	48	13 to 87	0.039	0.667	36	0.033	0.604
9	C16:0	—	—	—	—	—	38	0.047	0.730
10	C16 index	—	—	—	—	—	103	0.029	0.520
11	C5:0	3.76	97	0 to 124	0.006	0.127	97	0.010	0.200
11	C16:0	3.06	91	49 to 124	0.050	0.689	91	0.007	0.145
11	C10:1	4.03	49	23 to 118	0.017	0.320	49	0.007	0.147
11	C12:1	3.11	49	11 to 124	0.042	0.629	43	0.038	0.586
11	C10 index	3.88	75	49 to 124	0.047	0.665	80	0.015	0.284
11	C12 index	—	—	—	—	—	124	0.048	0.674
11	Fat %	—	—	—	—	—	124	0.024	0.421
13	C6:0	3.00	78	0 to 108	0.036	0.614	77	0.041	0.663
13	C12:1	2.99	59	0 to 101	0.050	0.739	—	—	—
13	C14:1	3.05	60	0 to 108	0.047	0.717	—	—	—
13	C16:1	3.47	75	17 to 108	0.011	0.258	75	0.006	0.146
13	C17:1	3.16	108	35 to 108	0.020	0.409	108	0.008	0.194
13	C12 index	2.98	56	0 to 108	0.045	0.698	101	0.043	0.684
13	C14 index	—	—	—	—	—	107	0.047	0.716
13	C16 index	2.94	101	0 to 108	0.035	0.607	104	0.026	0.502
13	C17 index	3.03	102	0 to 108	0.031	0.565	107	0.017	0.361
13	Fat %	3.63	53	11 to 69	0.008	0.197	53	0.016	0.352
14	C5:0	8.95	7	0 to 11	0.000	0.000***	—	—	—
14	C7:0	8.01	1	0 to 16	0.000	0.000***	—	—	—
14	C8:0	3.03	0	0 to 87	0.039	0.660	—	—	—
14	C9:0	9.29	1	0 to 8	0.000	0.000***	—	—	—
14	C11:0	7.97	1	0 to 5	0.000	0.000***	—	—	—
14	C13:0	5.56	3	0 to 88	0.000	0.008**	—	—	—
14	C14:0	9.84	0	0 to 0	0.000	0.000***	—	—	—

Continued

Table 2 (Continued). Location and characteristics of suggestive and significant QTL affecting short- and medium-chain fatty acids, unsaturation indices, and fat percentage

<i>Bos taurus</i> autosome	Trait	<i>F</i> -statistic	Position (cM)	95% Confidence interval (cM)	$P_{\text{chromosome}}$	P_{genome}	<i>DGAT1</i> and <i>SCD1</i> corrected ¹		
							Position (cM)	$P_{\text{chromosome}}$	P_{genome}
14	C15:0	6.28	2	0 to 25	0.000	0.000***	—	—	—
14	C16:0	13.86	0	0 to 1	0.000	0.000***	—	—	—
14	C10:1	—	—	—	—	—	49	0.028	0.540
14	C12:1	—	—	—	—	—	51	0.024	0.480
14	C14:1	—	—	—	—	—	58	0.045	0.714
14	C16:1	18.17	0	0 to 2	0.000	0.000***	—	—	—
14	C12 index	—	—	—	—	—	73	0.050	0.755
14	C14 index	4.55	0	0 to 99	0.001	0.032*	—	—	—
14	C16 index	10.19	0	0 to 16	0.000	0.000***	—	—	—
14	C17 index	3.36	0	0 to 73	0.014	0.325	0	0.049	0.749
14	Fat %	28.84	0	0 to 0	0.000	0.000***	—	—	—
16	C11:0	—	—	—	—	—	49	0.039	0.656
16	C10:1	3.35	19	1 to 88	0.047	0.727	18	0.025	0.486
16	C12:1	—	—	—	—	—	49	0.031	0.565
16	C12 index	—	—	—	—	—	52	0.029	0.551
17	C8:0	2.88	50	1 to 89	0.046	0.740	50	0.048	0.756
17	C10:1	3.53	28	0 to 95	0.025	0.513	—	—	—
19	C4:0	—	—	—	—	—	54	0.042	0.688
19	C7:0	—	—	—	—	—	0	0.040	0.669
19	C14:0	4.97	59	38 to 83	0.001	0.037*	57	0.002	0.061
19	C14:1	—	—	—	—	—	96	0.045	0.716
19	C16:1	2.85	54	14 to 96	0.044	0.702	—	—	—
19	C10 index	—	—	—	—	—	58	0.050	0.752
19	C12 index	—	—	—	—	—	96	0.003	0.079
19	C14 index	—	—	—	—	—	96	0.004	0.111
19	C16 index	—	—	—	—	—	96	0.035	0.622
21	C5:0	3.18	29	0 to 92	0.021	0.475	29	0.004	0.108
21	C7:0	3.22	29	0 to 92	0.018	0.423	29	0.001	0.042*
22	C10:0	—	—	—	—	—	39	0.044	0.789
22	C14:0	—	—	—	—	—	40	0.037	0.724
22	C15:0	3.44	52	10 to 76	0.014	0.390	55	0.015	0.407
22	C17:0	3.38	52	1 to 82	0.017	0.435	—	—	—
22	C17 index	—	—	—	—	—	61	0.024	0.558
23	C6:0	—	—	—	—	—	70	0.045	0.822
25	C4:0	3.16	56	8 to 63	0.018	0.555	56	0.018	0.549
26	C10:1	10.61	29	22 to 31	0.000	0.000***	—	—	—
26	C12:1	4.25	28	18 to 44	0.002	0.092	—	—	—
26	C14:1	9.52	28	22 to 37	0.000	0.000***	—	—	—
26	C16:1	6.04	31	19 to 35	0.000	0.000***	—	—	—
26	C17:1	3.82	32	17 to 64	0.002	0.084	—	—	—
26	C10 index	6.98	28	0 to 37	0.000	0.000***	—	—	—
26	C12 index	5.91	28	0 to 37	0.000	0.000***	—	—	—
26	C14 index	9.90	28	22 to 37	0.000	0.000***	—	—	—
26	C16 index	8.35	30	22 to 33	0.000	0.000***	—	—	—
26	C17 index	10.25	27	23 to 32	0.000	0.000***	—	—	—
27	C4:0	2.89	34	8 to 44	0.029	0.848	34	0.026	0.812
27	Fat %	2.58	33	15 to 44	0.049	0.959	—	—	—
29	C8:0	3.69	17	5 to 46	0.006	0.211	17	0.005	0.184
29	C10:0	3.35	17	6 to 66	0.019	0.570	17	0.016	0.490
29	C12:0	3.15	17	7 to 66	0.042	0.840	19	0.034	0.778

¹*DGAT1* = diacylglycerol acyltransferase 1; *SCD1* = stearoyl-coenzyme A desaturase 1.* $P_{\text{genome}} \leq 0.05$, ** $P_{\text{genome}} \leq 0.01$, *** $P_{\text{genome}} \leq 0.001$. All listed QTL have $P_{\text{chromosome}} \leq 0.05$.

Figure 1. Test statistic for genome-wide significant QTL for C6:0 and C8:0 on *Bos taurus* autosome (BTA) 6. The dotted black line represents the genome-wide significance threshold. This threshold may vary slightly between traits and is shown only for the trait with the lowest genome-wide significance level. The solid black line represents the information content. The triangles on the x-axis represent the locations of the markers.

average effect of 0.7 SD. The difference between the 2 daughter groups for C14:0 was 0.72 (wt/wt%) in family 2, 0.28 (wt/wt%) in family 4, and 1.14 (wt/wt%) in family 7. This QTL explained 4% of phenotypic variance in C14:0.

On BTA26, a QTL near the location of the *SCD1* gene (28 cM) affected all monounsaturated FA and their unsaturation indices (Table 2, Figure 4). The size of the QTL effect was about 0.7 phenotypic SD for all traits (Table 3). The allele that positively affected C10, C12, and C14 FA had a negative effect on C16 and C17 FA. Sires of families 1, 2, and 6 were heterozygous. In families 1 and 2, the QTL significantly affected all monounsaturated FA and unsaturation indices. Family 6 had only 29 offspring; therefore, not all QTL effects were significant. This QTL explained between 4 and 8% of phenotypic variance.

In addition to the significant QTL, 49 traits with suggestive QTL were found on 21 autosomes (Table 2). No QTL was found on BTA10, BTA12, BTA15, BTA18, BTA20, BTA23, BTA24, or BTA28. Although QTL were suggestive, some of these chromosomal regions

were found for multiple traits that are known to overlap in synthesis pathways. For short, even-chain FA, suggestive QTL were found on 2 chromosomes; on BTA2 for C4:0 and C6:0, and on BTA29 for C8:0, C10:0, and C12:0. For odd-chain FA, suggestive QTL were found on 2 other chromosomes: on BTA21 for C5:0 and C7:0, and on BTA22 for C15:0 and C17:0. For several mono-unsaturated FA and unsaturation indices, there were suggestive QTL on BTA5, BTA11, and BTA13.

Effect of *DGAT1* and *SCD1* Genotype Polymorphisms

In a second analysis, data were adjusted for the effects of the *DGAT1* K232A and *SCD1* A293V genotypes, which resulted in complete disappearance of all linkage previously found on BTA14 and BTA26, except for the suggestive QTL for the C17 index on BTA14 (Table 2). This means that the detected QTL were most likely caused by the known mutations in *DGAT1* and *SCD1*. After accounting for known mutations, however, a new suggestive QTL was found on BTA14 for C10:1, C12:1, and C14:1 at approximately 50 cM, and for the C12

Table 3. Allele substitution effects and standard errors (subscript) within 7 paternal half-sib families for QTL on *Bos taurus* autosome (BTA) 6, BTA14, BTA19, and BTA26, and approximate phenotypic variation explained by QTL

Trait	Family (no. of daughters)							Phenotypic variation explained by QTL (%)
	1 (193)	2 (179)	3 (170)	4 (166)	5 (91)	6 (29)	7 (21)	
BTA6								
C6:0	−0.08 _{0.02} *	0.03 _{0.02}	−0.03 _{0.02}	−0.01 _{0.02}	−0.05 _{0.03}	−0.10 _{0.05} *	0.04 _{0.06}	3
C8:0	−0.07 _{0.02} *	0.02 _{0.02}	−0.01 _{0.02}	−0.01 _{0.02}	−0.07 _{0.02} *	−0.03 _{0.04}	0.05 _{0.05}	3
BTA14								
Fat %	−0.53 _{0.09} *	−0.69 _{0.09} *	−0.70 _{0.10} *	−0.68 _{0.10} *	−0.06 _{0.18}	0.50 _{0.33}	−0.93 _{0.26} *	19
C5:0	−0.01 _{0.00} *	0.00 _{0.00} *	−0.01 _{0.00} *	−0.01 _{0.00} *	−0.01 _{0.00}	0.00 _{0.01}	−0.01 _{0.01}	7
C7:0	−0.01 _{0.00} *	−0.01 _{0.00} *	−0.01 _{0.00} *	−0.01 _{0.00} *	−0.01 _{0.00}	0.01 _{0.01}	0.00 _{0.01}	6
C9:0	−0.01 _{0.00} *	0.00 _{0.00}	−0.01 _{0.00} *	−0.01 _{0.00} *	−0.01 _{0.01}	0.02 _{0.01}	−0.01 _{0.01}	7
C11:0	−0.01 _{0.00} *	−0.01 _{0.00}	−0.02 _{0.01} *	−0.02 _{0.01} *	−0.01 _{0.01}	0.02 _{0.02}	−0.01 _{0.01}	6
C14:0	0.43 _{0.11} *	0.59 _{0.11} *	0.35 _{0.12} *	0.35 _{0.12} *	−0.01 _{0.23}	0.65 _{0.41}	0.92 _{0.32} *	7
C15:0	−0.09 _{0.03} *	−0.03 _{0.03}	−0.11 _{0.03} *	−0.11 _{0.03} *	−0.10 _{0.05}	0.05 _{0.10}	−0.07 _{0.08}	5
C16:0	−1.63 _{0.33} *	−1.57 _{0.34} *	−1.34 _{0.36} *	−1.67 _{0.35} *	0.03 _{0.66}	−1.39 _{1.19}	−3.25 _{0.92} *	10
C16:1	−0.21 _{0.04} *	−0.30 _{0.04} *	−0.13 _{0.05} *	−0.32 _{0.05} *	0.13 _{0.09}	−0.19 _{0.16}	−0.20 _{0.12}	13
C16 index	−0.38 _{0.11} *	−0.63 _{0.12} *	−0.16 _{0.13}	−0.61 _{0.12} *	0.39 _{0.23}	−0.44 _{0.42}	−0.15 _{0.32}	8
C13:0	−0.01 _{0.01} *	−0.01 _{0.01}	−0.02 _{0.01} *	−0.02 _{0.01} *	−0.01 _{0.01}	0.02 _{0.02}	−0.01 _{0.02}	4
C14 index	−0.65 _{0.25} *	−0.83 _{0.26} *	−0.40 _{0.28}	−0.87 _{0.27} *	0.51 _{0.52}	−0.70 _{0.93}	−0.80 _{0.72}	3
BTA19								
C14:0	0.09 _{0.10}	0.72 _{0.22} *	−0.20 _{0.11}	−0.28 _{0.11} *	0.08 _{0.15}	−0.32 _{0.30}	1.14 _{0.33} *	4
BTA26								
C10:1	0.05 _{0.01} *	0.04 _{0.01} *	0.01 _{0.01}	0.01 _{0.01}	0.01 _{0.01}	0.03 _{0.02}	−0.03 _{0.02}	8
C14:1	0.18 _{0.03} *	0.16 _{0.03} *	0.00 _{0.04}	0.03 _{0.04}	0.08 _{0.05}	0.21 _{0.09} *	0.04 _{0.09}	6
C16:1	−0.22 _{0.04} *	−0.15 _{0.04} *	0.03 _{0.05}	0.04 _{0.05}	0.08 _{0.06}	−0.04 _{0.11}	0.06 _{0.12}	5
C10 index	1.26 _{0.23} *	0.93 _{0.24} *	0.04 _{0.26}	0.30 _{0.26}	0.44 _{0.34}	0.87 _{0.64}	−0.24 _{0.69}	5
C12 index	0.30 _{0.07} *	0.26 _{0.07} *	0.01 _{0.08}	0.09 _{0.08}	0.15 _{0.10}	0.34 _{0.19}	0.22 _{0.20}	4
C14 index	1.29 _{0.23} *	1.14 _{0.23} *	0.03 _{0.25}	0.30 _{0.25}	0.69 _{0.32} *	1.51 _{0.62} *	0.45 _{0.67}	6
C16 index	−0.62 _{0.11} *	−0.45 _{0.11} *	0.07 _{0.12}	0.17 _{0.11}	0.29 _{0.15}	−0.14 _{0.29}	0.17 _{0.31}	6
C17 index	−2.31 _{0.36} *	−1.69 _{0.37} *	0.19 _{0.39}	0.79 _{0.39} *	0.93 _{0.51}	−1.04 _{0.97}	0.79 _{1.06}	8

*Significantly segregating QTL ($P \leq 0.05$, calculated by a t -test).

index at 73 cM. This suggestive QTL was also detected for several long-chain monounsaturated FA (see Schenink et al., 2009). Furthermore, precorrection for the *DGAT1* and *SCD1* genotypes resulted in additional suggestive QTL for the unsaturation indices of C10 to C16 on BTA19 and a significant QTL for C7:0 on BTA21. In total, 39 traits no longer showed significant linkage when correcting for *DGAT1* and *SCD1*, of which 23 traits were located on BTA14 and BTA26, whereas 29 new traits with suggestive QTL were identified.

DISCUSSION

Power Calculations

In this study, we reported on suggestive QTL with $P_{\text{chromosome}} < 0.05$ and significant QTL with $P_{\text{genome}} < 0.05$. Based on power calculations on a daughter design of this size (Weller et al., 1990), there was a high (>80%) power to identify QTL that explained at least 5% of phenotypic variance and a medium (about 50%) power to identify QTL that explained 2.5 to 4.2% of phenotypic variance. The significant QTL found in this

study explained 3 to 19% of phenotypic variance, which is in accordance with this expectation.

Adjusting the phenotypic data for effects of known genes reduces the residual variation in the traits and is therefore expected to result in a higher power to detect QTL (De Koning et al., 2001). In a second analysis, the phenotypic data were precorrected for the *DGAT1* K232A and *SCD1* A293V mutations. Several new suggestive QTL and 1 new significant QTL were detected. For some FA, including the previously found QTL on BTA6 and BTA19, the test statistic decreased below the threshold for suggestive QTL. In general, the change in test statistic was rather small (<0.3).

Fat Percentage

In contrast to the limited research on FA proportions, fat percentage and fat yield have been studied extensively. A review by Khatkar et al. (2004) reported significant QTL for fat percentage and fat yield on BTA2, BTA3, BTA4, BTA6, BTA9, BTA10, BTA12, BTA14, BTA16, BTA20, and BTA26. The current study found one highly significant QTL for fat per-

Figure 2. Test statistic for genome-wise significant QTL for saturated short- and medium-chain fatty acids on *Bos taurus* autosome (BTA) 14. The dotted black line represents the genome-wise significance threshold. This threshold may vary slightly between traits and is shown only for the trait with the lowest genome-wise significance level. The solid black line represents the information content. The triangles on the x-axis represent the locations of the markers.

centage on BTA14 and 4 suggestive QTL on BTA6, BTA8, BTA13, and BTA27. The suggestive QTL on BTA6 is also mentioned in the review by Khatkar et al. (2004).

The current study found 4 significant QTL for short- and medium-chain FA on BTA6, BTA14, BTA19, and BTA26, and numerous suggestive QTL, which do not match the locations of the QTL for fat percentage that were detected. Finding different QTL for fat percentage and milk-fat composition was partly due to more detailed phenotyping of individuals. Milk-fat composition is not the same trait as milk-fat percentage. Although there is obviously a relationship between fat percentage and milk-fat composition, the genetic correlations between individual FA proportions and fat percentage differ largely between FA and range between 0.00 and 0.78 (Stoop et al., 2008). Some FA proportions increase with increasing fat percentage, whereas others decrease. These differences in size and sign of genetic correlations imply several genetic and physiological mechanisms with opposing effects, which might explain the presence

of QTL for milk-fat composition that do not affect fat percentage.

Fatty Acid Synthesis

Several other studies have looked at candidate genes that were expected to play a role in milk-fat synthesis, such as *PPARGC1A* (Khatib et al., 2007), *FAM13A1* (Cohen et al., 2004), and *OPN* on BTA6 (Schnabel et al., 2005; Khatib et al., 2007); *DGAT1* on BTA14 (Schennink et al., 2007); *GH* (Viitala et al., 2006), *FASN* (Smith, 1994), and *STAT5A* on BTA19 (Stephens et al., 1999; Brym et al., 2004); and *SCD1* on BTA26 (Schennink et al., 2008). Although most studies reported effects on fat percentage, data on milk-fat composition were not available, except for those of Schennink et al. (2007, 2008). In fact, only very few studies have reported effects on fat composition, mainly in adipose tissue of beef cattle (Reh et al., 2004; Taniguchi et al., 2004; Moiola et al., 2007; Morris et al., 2007; Zhang et al., 2008).

Figure 3. Test statistic for genome-wise significant QTL for C14:0 on *Bos taurus* autosome (BTA) 19. The dotted black line represents the genome-wise significance threshold. The solid black line represents the information content. The triangles on the x-axis represent the locations of the markers.

Strong evidence was found for significant QTL on BTA14 and BTA26. These QTL effects are likely caused by *DGAT1* (BTA14) and *SCD1* (BTA26), because these QTL disappear after correction for the *DGAT1* and *SCD1* genotypes. The C10 to C18 FA can be desaturated by *SCD1* (Enoch et al., 1976; Chung et al., 2000), and sequentially, all present individual FA can be esterified to glycerol via GPAT (Roy et al., 2006a), AGPAT (Mistry and Medrano, 2002), and *DGAT1* (Winter et al., 2002) to end up as triacylglycerols in the milk.

A significant QTL for C6:0 and C8:0 was found on BTA6 at approximately 55 cM, which is in near proximity to the *PPARGC1A* gene. The *PPARGC1A* gene is one of several major transcription factors that has been proposed to play a central role in regulation of milk-fat synthesis activity in the mammary gland (Khatib et al., 2007; Bernard et al., 2008). The *PPARGC1A* gene is involved in up- and downregulation of several genes of the acetyl-CoA cycle, such as acetyl-CoA carboxylase, which activates acetyl-CoA (ACC; Barber et al., 1997; Mao et al., 2001) and fatty acid synthase (FASN; Smith, 1994).

A significant QTL for C14:0 was found on BTA19. Morris et al. (2007) reported a significant effect of the candidate gene *FASN* (BTA19) on C14:0. Within the acetyl-CoA cycle, chain elongation is initiated by acyl-transferase, 1 of the 6 enzymes associated with FASN, which loads mainly acetyl and malonyl substrates to β -ketoacylsynthase (Roy et al., 2005). Regulation of chain termination for C14:0 and C16:0 is done by thioesterase I, another enzyme associated with FASN (Smith, 1994). The *FASN* gene, in particular the thioesterase I enzyme, might be a good candidate for the detected QTL on BTA19 in the present study. However, at least 2 other genes in the proximity of the QTL have been associated with fat synthesis, and could also be responsible for the observed QTL effect: *GH* and *STAT5A* (Brym et al., 2004; Viitala et al., 2006). The *STAT5* gene mediates a prolactin signal and stimulates promoter III of acetyl-CoA carboxylase (Rosen et al., 1999; Mao et al., 2002), and can in turn be stimulated by GH and IGF1 (Yang et al., 2000).

Several QTL were identified for short- or medium-chain FA: for C4:0 and C6:0, a suggestive QTL was found on BTA2, for C6:0 and C8:0 on BTA6, for C14:0

Figure 4. Test statistic for genome-wide significant QTL for unsaturation indices on *Bos taurus* autosome (BTA) 26. The dotted black line represents the genome-wide significance threshold. This threshold may vary slightly between traits and is shown only for the trait with the lowest genome-wide significance level. The solid black line represents the information content. The triangles on the x-axis represent the locations of the markers.

on BTA19, and for C8:0, C10:0, and C12:0 on BTA29 (suggestive). Although these chromosomes harbor genes that have been related to milk-fat synthesis, additional mapping at higher resolutions will be necessary to reduce the size of confidence intervals and identify possible candidate genes. Increasing the number of SNP, especially in currently sparse regions such as the 70 to 95 cM region on BTA19, will improve the information content of the linkage map.

CONCLUSIONS

Four distinct significant QTL were identified that affected short- and medium-chain FA. Two QTL affected C6:0 and C8:0 (BTA6) and C14:0 (BTA19). The other 2 QTL were most likely caused by *DGAT1* (BTA14) and *SCD1* (BTA26). These 4 QTL explain 3 to 19% of phenotypic variance in the affected traits. In total, 49 suggestive QTL were reported that affect either even-chain FA, odd-chain FA, or monounsaturated FA and unsaturation indices.

Quantitative trait loci that affect specific FA might increase the understanding of physiological processes

involved in fat synthesis and may aid in locating the underlying genes. Selection might be used to decrease proportions of unfavorable FA in bovine milk, such as C16:0, and reduce the fat melting point for more spreadable butter.

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