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Association of *DGAT1* genotype, fatty acid composition, and concentration of copper in milk with spontaneous oxidized flavor

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ABSTRACT

In 136 cows with altogether 969 milk samples, we investigated the effect of the acyl-coenzyme A:diacylglycerol acyltransferase 1 (*DGAT1*) *K232A* polymorphism on milk fatty acid (FA) composition and how, in combination with copper concentration in milk, this influences the occurrence of spontaneous oxidized flavor. All milk samples were analyzed for concentrations of copper and individual FA and subjected to sensory analysis by trained judges. We found an effect of *DGAT1* genotype on FA composition where mainly the long-chain FA were affected. The *232A* allele was associated with larger proportions of the C18:2 *cis*-9,*trans*-11 conjugated linoleic acid and lower proportions of C16:0 FA. Milk concentrations of unsaturated FA and copper showed strong and unfavorable associations with spontaneous oxidized flavor (SOF) development. The interaction between FA and copper indicates that SOF will not develop as easily in milk with high copper content unless the substrate is available (i.e., in addition to the previously shown effect of copper in milk, unsaturated FA are required for the process of oxidation to progress). We observed a marked effect of the *DGAT1* genotype on SOF development where the *A* allele was associated with a higher risk of SOF. Moreover, our results suggest that the effects of the FA C18:3 n-3 and of the polyunsaturated index on SOF development are beyond the effect of the *DGAT1* genotype. Breed had an effect on FA composition but not on SOF development. Our results imply that copper, FA composition, and *DGAT1* genotype are risk factors for SOF and considerations to these factors might be necessary in future breeding decisions.

Key words: spontaneous oxidized flavor, fatty acid composition, copper, *DGAT1*

INTRODUCTION

Milk fat composition has a major influence on dairy products affecting shelf life and processing quality of the milk. A more unsaturated milk fat is preferred from human nutritional and health perspectives. The FA composition in milk varies due to factors such as feed, stage of lactation, parity, season, and genotype of the cow (see review by Palmquist, 2006). Several studies have identified genetic variation in the composition of milk fat (Karijord et al., 1982; Soyeurt et al., 2007; Bobe et al., 2008; Stoop et al., 2008; Garnsworthy et al., 2010), and efforts are being made to elucidate the genes contributing to this variation. Milk fat consists of approximately 98% triglycerides, and the acyl-CoA:diacylglycerol acyltransferase 1 (**DGAT1**) enzyme has an important function in milk fat synthesis, because it catalyzes the final step in the formation of triglycerides. A dinucleotide substitution in the gene coding for DGAT1, resulting in a replacement of the AA lysine (K) with alanine (A) in position 232 (*K232A*), has been shown to be associated with increased yields of protein and milk, and a decrease in yield of fat and concentrations of fat and protein (Grisart et al., 2002; Spelman et al., 2002; Winter et al., 2002; Thaller et al., 2003). In a study by Schennink et al. (2007), it was found that the *DGAT1 K* allele was associated with a larger fraction of C16:0 and smaller fractions of C14:0 and unsaturated C18. The same research group also reported evidence that the *DGAT1 A* allele is associated with a higher unsaturation index for the long-chain FA found in milk, and argued that selective breeding in favor of the *A* allele would give milk with a more desirable FA composition, from a public health perspective (Schennink et al., 2008).

A potential downside of such a breeding strategy is that unsaturated milk FA are more prone to oxidize (Sidhu et al., 1975; Barrefors et al., 1995; Granelli et al., 1998; Timmons et al., 2001), giving a carbon, metal, talcum, or fishy flavor to the milk (Shipe et al., 1978). This off-flavor results from volatile compounds that accumulate in the milk through the oxidation of the double bonds between the carbon atoms in unsatu-

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rated FA. Oxidative off-flavor can be either induced or spontaneous (**SOF**; i.e., develop without exposure to light and with no addition of prooxidants). Oxidation is often initiated by naturally occurring prooxidants such as copper and iron, but to a certain extent these can be balanced by antioxidative substances in the milk, such as β-carotene and α-tocopherol. According to the report by Lindberg et al. (2004), oxidative off-flavor is the second most prevalent off-flavor in bulk milk at individual farms, next to rancid flavor. Of the routine bulk milk samples collected monthly from each farm for sensory tests during year 2002, 1.81% had an off-flavor, of which 0.39% were judged to have an oxidative off-flavor, compared with 0.65% of samples with rancid flavor. Variation in oxidative stability of milk from individual cows has previously been reported by Corbett and Tracy (1943), Dunkley and Franke (1967), Barrefors et al. (1995), and Granelli et al. (1998). In a recent publication, Clausen et al. (2010) observed large individual variation in oxidative stability of milk and concluded that FA composition and antioxidants such as α-tocopherol only explained a limited part of the variation in milk oxidation. Comparable results were reported by Juhlin et al. (2010a) regarding α-tocopherol, whereas the latter study found that concentration of PUFA in milk was positively associated with increased risk of developing SOF. The most potent prooxidant in milk is copper (Haase and Dunkley, 1970; Bruhn et al., 1975). Copper occurs naturally in milk where its concentration varies between cows and also depends on the diet and level of mineral supplementation (Dunkley et al., 1968). We have previously shown that copper concentration in milk has a major effect on the development of SOF (Juhlin et al., 2010a,b).

The dairy herd at the Swedish University of Agricultural Sciences includes cows of the Swedish Red (**SR**) and the Swedish Holstein (**SH**) breeds. The SR cows belong to either of 2 selection lines producing milk with a high or low concentration of milk fat at equal total milk energy production, such that cows from both lines have similar nutritional requirements. This material offers an interesting opportunity to explore the biology and genetics behind variation in milk composition and especially fat content. The aim of the present study was to investigate the effect of a *DGAT1* polymorphism and selection toward high or low milk fat content on milk FA composition and how this, in combination with natural variation in milk copper content, influences the occurrence of SOF. Due to the proposed relationship between *DGAT1* genotype and the proportion of unsaturated FA in milk (Schennink et al., 2007) we hypothesize that the *DGAT1 K232A* polymorphism is associated with the tendency of milk to develop SOF.

MATERIALS AND METHODS

Animals

The experimental herd at the University of Agricultural Sciences in Uppsala, Sweden consists of pure bred dairy cows of the SR and the SH breeds. Since 1985, the SR cows have been selected for either high (**HF**) or low (**LF**) milk fat content, with the 2 lines having an equal total milk energy production. The cows in the 2 lines are inseminated with bulls with high or low breeding values for milk fat content, respectively, all of them belonging to the top bulls for total milk energy production. The bulls used for insemination of the SH cows rank among the top bulls, based on total merit index. A total of 136 cows from the experimental herd were available for this study of which 50 belonged to the SH breed, 38 belonged to the SR/HF line, and 48 to the SR/LF line. The cows were genotyped for the *DGAT1 K232A* polymorphism using the method of pyrosequencing (Ronaghi et al., 1998) according to Näslund et al. (2008).

Sampling and Analysis of Milk

Individual morning milk samples were collected monthly from all lactating cows in the herd from October 2000 until December 2001 (a total of 969 samples). Depending on the number of months in lactation during this period, the number of samples per cow varied between 1 and 11. Fat, protein, and lactose concentrations were determined by infrared spectroscopy (Dairy-Lab2, A7S; Foss Electric A/S, Hillerød, Denmark). Aliquots of morning milk were stored at −80°C for analysis of FA composition. Lipids were extracted according to the method described by Nourooz-Zadeh and Appelqvist (1988). Preparation of FA methyl esters was done using the protocol by Appelqvist (1968), and analysis was carried out with a HP5890 series II gas chromatograph (Hewlett Packard Co., Rolling Meadows, IL), fitted with a flame ionization detector and a capillary column DB-23 (Agilent Technologies, Stockholm, Sweden; 30-m length, 0.25-mm i.d., 0.25-μm film thickness). The column temperature was programmed at 2°C/min from 40 to 220°C. Injector and detector temperatures were 250°C. Identification of FA was performed by comparing the obtained peaks with those of standards (Larodan Fine Chemicals; Sigma Chemical Co., Malmö, Sweden). Peak areas were integrated using HP ChemStation software. The carrier gas was helium, and make-up gas was nitrogen. Values are shown for the major milk FA and those with particular relevance for fat oxidation (Table 1). The methylation step used for the FA analyses was

not optimized for short-chain FA, which influenced the accuracy of these measures. Therefore, concentrations of the short-chain FA are not shown. Analysis of copper content in milk was performed as described in Juhlin et al. (2010b).

Aliquots of morning milk were tested for sensory quality by trained judges. In this test, each sample was evaluated according to an instruction manual that carefully describes the sensory parameters to be considered, how to handle samples, and how judges should be trained. The same 2 judges tested each milk sample, independently of each other. Odor and taste were scored according to a standard of the expected quality characteristics of normal Swedish milk and deviations from the standard as described in the instruction manual (G. Virdeskog, Eurofins Steins Laboratory AB, Jönköping, Sweden, personal communication). The milk samples were classified as either normal, moderate off-flavor (class 1B), or pronounced off-flavor (class 2) with regard to SOF. To classify a milk sample as belonging to class 1B, 1 of the 2 judges must have smelled or tasted (or both) an abnormal odor/flavor in the milk, whereas if both persons characterized the off-flavor as pronounced, the milk was assigned to class 2. The judges were trained in recognizing the off-flavors included in the Swedish test system. Statistics regarding the tests performed throughout the year were analyzed to investigate the judges' performance and sensitivity to the different off-flavors. Throughout the year, the laboratory organized sessions with all judges, using known samples of different grades of off-flavor, to harmonize the assessments between judges and thereby reduce bias.

Statistical Analysis

Effect of *DGAT1* genotype on FA composition was analyzed using the PROC MIXED (SAS Institute Inc., Cary, NC) with the model below (model 1):

$$y_{ijklmnopq} = \mu + s_i + parity_j + group_k + gen_l + parity \times f(DIM)_{mn} + cow_{op} + a_{op} + e_{ijklmnopq},$$

where $y_{ijklmnopq}$ = the test-day record measured on cow_{op}, μ = intercept, s_i = effect of season of testing ($i = 1, 2, \ldots, 4$), $parity_j$ = the effect of parity number ($j = 1, 2, \ldots, 6$), $group_k$ = the effect of group (k = SH, SR/HF, or SR/LF), gen_l = effect of *DGAT1* genotype (l = *AA* and *AK*), $f(DIM)_{mn}$ = denotes the 5-parameter Ali and Schaeffer (1987) function describing the average shape of a lactation curve of cows, cow_{op} = random environmental effect of cow, a_{op} = random additive genetic effect of polygenic background of cow_{op}, and $e_{ijklmnopq}$ = the random residual effect.

Table 1. Proportions of major FA and copper concentration of 969 monthly morning milk samples from cows of the Swedish Holstein breed and 2 selection lines[1] of the Swedish Red breed

Trait[2]	Mean	SD
C14:0	13.59	2.40
C15:0	1.64	3.93
C16:0	36.58	5.23
C17:0	0.75	1.33
C18:0	10.88	2.32
cis 18:1 n-9	25.93	4.77
C18:2 n-6	2.17	0.45
C18:3 n-3	0.63	0.18
CLA	0.74	0.47
PUFA	3.43	0.71
PI	4.03	0.82
Copper, μg/kg	50.12	39.30

[1]Cows from selection lines for high fat content (HF; 38 cows) or low fat content (LF; 48 cows), respectively, but with similar total milk energy production. SH = 50 cows.

[2]Fatty acids and groups of FA are expressed as g/100 g of FA. CLA = conjugated linoleic acid (refers to *cis*-9,*trans*-11 C18:2); PUFA = C18:2 n-6 + C18:3 n-3 + CLA; PI = polyunsaturated index [C18:2 n-6 + (C18:3 n-3 × 2)].

An additive polygenic background effect, accounted for by a relationship matrix with up to 2 generations of ancestors, was included to avoid bias in the estimates of *DGAT1 K232A* genotype effects. Parity and DIM were used as repeated measures variables with the SAS SP(POW) structure. Observations adjusted for fixed effects from these analyses (residual values, hereinafter referred to as adjusted values) were stored for use in subsequent analysis.

The effects of milk concentrations of copper, different categories of fat, and their interaction on occurrence of SOF were investigated using PROC GLIMMIX (SAS Institute Inc.). The individual PUFA C18:2 n-6, C18:3 n-3, and conjugated linoleic acid (**CLA**; *cis*-9,*trans*-11 C18:2) were included in the statistical analysis. Similar to the study by Timmons et al. (2001), the PUFA were grouped into total PUFA (C18:2 n-6 + C18:3 n-3 + CLA) and polyunsaturated index [**PI**; C18:2 n-6 + (C18:3 n-3 × 2)], of which the latter accounts for the number of pentadienyl groups. Analyzing these combinations of FA reflects the hypothesis that the higher the PI or the more PUFA, the more SOF, irrespective of the relative contribution of the individual unsaturated FA to PUFA and PI. Only one measure of category of fat (C18:2 n-6, C18:3 n-3, CLA, PUFA, or PI) at a time was included in the model. These effects as well as the effect of breed and selection line were offered to the model but only significant ones ($P < 0.05$) were kept, yielding the following model for analyzing the variation in SOF (model 2):

$$\log\left(\frac{\pi_{ijk}}{1-\pi_{ijk}}\right) = \mu_r + b_1 \cdot \text{logcopper} + b_2 \cdot \text{fat} + b_3 \cdot \text{logcopper} \times \text{fat} + a_j + pe_k,$$

where π_{ijk} = probability that the test-day SOF falls in category r ($r = 1$, 2, or 3); μ_r = intercept; b_1, b_2, and b_3 = regression coefficients; logcopper = the logarithm of copper concentration; fat = concentration of different categories of fat; a_j = random additive genetic effect of polygenic background of cow; and pe_k = random environmental effect of cow.

To test for the *DGAT1* genotype effect on SOF development (model 3), model 2 was modified such that the main and interactions effects of the different categories of fat were replaced by an effect of the *DGAT1* genotype and an interaction between genotype and logcopper:

$$\log\left(\frac{\pi_{ijk}}{1-\pi_{ijk}}\right) = \mu_r + b_1 \cdot \text{logcopper} + b_2 \cdot \text{DGAT1} + b_3 \cdot \text{logcopper} \times \text{DGAT1} + a_j + pe_k.$$

Last, to further improve the analyses of the variation in SOF, a model including copper, *DGAT1* genotype, and categories of fat as explanatory variables was tested (model 4). The interactions between the different categories of fat and logcopper as well as between *DGAT1* genotype and logcopper were also included. In this model 4, we used the adjusted values from the PROC MIXED (model 1) analysis to avoid confounding due to the association between *DGAT1* polymorphism and FA composition; that is, AdjFat denotes the concentration of the (index of) FA adjusted for *DGAT1* polymorphism and other fixed effects:

$$\log\left(\frac{\pi_{ijk}}{1-\pi_{ijk}}\right) = \mu_r + b_1 \cdot \text{logcopper} + b_2 \cdot \text{DGAT1} + b_3 \cdot \text{logcopper} \times \text{DGAT1} + b_4 \cdot \text{adjFat} + b_5 \cdot \text{log copper} \times \text{adjFat} + a_j + pe_k.$$

Table 2. The *DGAT1* genotypes of 136 cows of the Swedish Holstein breed (SH) and 2 selection lines of the Swedish Red breed (SR)

Breed/ selection line[1]	*DGAT1* genotype		
	AA	*AK*	*KK*
SR/HF	24	14	—
SR/LF	46	2	—
SH	40	10	—
Total	110	26	—

[1]Cows from selection lines for high fat content (HF) or low fat content (LF), respectively, but with similar total milk energy production.

As in model 1, a relationship matrix was included in model 2 to account for the random, additive genetic effect of the polygenic background, and in model 4 to reduce bias in the estimates of *DGAT1* genotype effects. The SP(POW) structure, with parity and DIM as repeated measures, was used to account for the repeated observations on cows.

RESULTS AND DISCUSSION

As expected, the average milk fat content was higher in the SR/HF cows (4.8%) compared with the SR/LF (4.0%) and the SH (3.8%) cows. Composition of milk fat was within the range of what has been reported in previous studies (Jensen, 2002) and the mean copper concentration found in this study was (mean ± SD) 50.12 ± 39.30 μg/kg (Table 1), which is also within the range of what other studies have found (Bruhn et al., 1975; Ford et al., 1986; Sol Morales et al., 2000; Timmons et al., 2001; Havemose et al., 2006).

Genotype and Allele Frequencies

The *DGAT1 AA* genotype was the most common in this material, whereas the *KK* genotype was not present (Table 2). We had previously reported a high frequency of the *A* allele in 239 cows of which the present group was a part (Näslund et al., 2008) where we suggested that selection for a lower fat content in milk is an indirect selection for the *A* variant. For decades, the breeding strategies in Sweden have given the *A* variant a selective advantage by favoring milk volume rather than DM content, which might be a reason for the high frequency of this variant in the present material. The cows selected for low fat content in milk had a lower frequency of the *AK* genotype compared with the cows selected for high fat content (4 and 37%, respectively), which is in line with previous studies (e.g., Grisart et al., 2002; Winter et al., 2002) in which the *K* allele has been associated with high fat content and the *A* allele with low fat content.

FA Composition

The proportion of individual FA differed between the SH and the SR groups of cows (Table 3). Milk from the SH cows had higher amounts of all measured C18 PUFA, analyzed individually or pooled, compared with the SR cows, the only exception being CLA for which both the SR/LF and the SH cows had higher amounts than the SR/HF cows. Differences between breeds concerning milk FA composition have been reported previously, with Holstein cows generally producing milk with larger proportions of the C18 unsaturated FA

Table 3. Differences (±SE) in proportion of some FA in 969 morning milk samples between cows of the Swedish Holstein breed (SH) and 2 selection lines of the Swedish Red breed (SR)

	Group[2]		
Trait[1]	SR/HF	SR/LF	SH
C14:0	0[a]	−0.510 ± 0.290[b]	−0.960 ± 0.250[c]
C16:0	0[a]	−0.628 ± 0.546[a]	−0.748 ± 0.472[a]
C18:0	0[a]	−0.293 ± 0.307[a]	0.323 ± 0.268[a]
cis C18:1 n-9	0[a]	−0.292 ± 0.300[a]	−0.320 ± 0.268[b]
C18:2 n-6	0[a]	0.056 ± 0.064[a]	0.250 ± 0.056[b]
CLA	0[a]	0.046 ± 0.029[b]	0.049 ± 0.025[b]
C18:3 n-3	0[a]	0.000 ± 0.023[a]	0.036 ± 0.020[b]
PUFA	0[a]	0.086 ± 0.110[a]	0.260 ± 0.090[b]
PI	0[a]	0.069 ± 0.135[a]	0.278 ± 0.112[b]

[a–c]Values within a row with different superscripts differ ($P < 0.05$).

[1]Fatty acids and groups of FA are expressed as g/100 g of FA. CLA = conjugated linoleic acid (refers to *cis*-9,*trans*-11 C18:2); PUFA = C18:2 n-6 + C18:3 n-3 + CLA; PI = polyunsaturated index [C18:2 n-6 + (C18:3 n-3 × 2)].

[2]Group contains information on breed and selection line. Cows from selection lines for high fat content (HF; 38 cows) or low fat content (LF; 48 cows), respectively, but with similar total milk energy production. SH = 50 cows.

(Krukovsky, 1961; Stull and Brown, 1964; Palmquist and Beaulieu, 1992; Arnould and Soyeurt, 2009). Furthermore, the concentration of the SFA C14 (and a tendency in C16) was lower in the LF cows (Table 3). These results are in agreement with previous studies in which estimated heritabilities and genetic correlations indicate that selection toward high milk fat content would lead to an increased proportion of SFA of medium length and a lower proportion of unsaturated C18 FA (Renner and Kosmack, 1974; Karijord et al., 1982; Soyeurt et al., 2007; Stoop et al., 2008).

The *DGAT1* genotype contributed to the variation in FA composition in our study, and the effect was the same across selection lines and breeds, reflected in a nonsignificant genotype × group interaction effect ($P > 0.1$). However, it should be noted that the lack of interaction may also be due to the small numbers of animals in each of the breed groups and genotype combination (Table 2). The *K* allele was associated with a larger proportion of C16, a result supported by Schennink et al. (2007). They also found that the *K* allele was associated with a larger proportion of C14, which was not, however, found in our study. In the present study, the *K* allele showed a tendency of being associated with relatively low proportions of all unsaturated FA included in the analysis (Table 4). Schennink et al. (2007) also reported an association of the *K* allele with a lower proportion of unsaturated C18 FA.

The genetic component accounted for by the relationship matrix was significant for all FA analyzed ($P < 0.01$). Due to the relatively small data set, neither heritability nor repeatability estimates were presented. However, previous studies report a decreasing trend in heritabilities of individual FA concentrations with increasing length of the carbon chain, a trend that was also observed by Stoop et al. (2008). Also Schennink et al. (2007) found high heritabilities for short-chain FA and medium-chain FA (0.43 to 0.59), but lower estimates for the long-chain FA (around 0.25), with an exception for CLA (0.42).

Spontaneous Oxidized Flavor

Of 969 milk samples analyzed, 8% had a moderate SOF and 4% a high SOF. Compared with the prevalence of SOF in bulk milk at individual farms (Lindberg et al., 2004) these figures were considerably higher. A

Table 4. Effect of *DGAT1 K232A* genotype (solutions expressed as the difference between *AA*-*KA* genotypes) on proportions (%) of FA in milk from cows of the Swedish Holstein breed and 2 selection lines[1] of the Swedish Red breed

Trait[2]	Effect[3]	SE	*P*-value
C14:0	0.244	0.232	0.29
C16:0	−1.211	0.501	0.011
C18:0	−0.399	0.292	0.14
cis C18:1 n-9	0.944	0.550	0.087
C18:2 n-6	0.085	0.041	0.070
CLA	0.068	0.026	0.010
C18:3 n-3	0.027	0.017	0.13
PUFA	0.142	0.078	0.092
PI	0.157	0.086	0.12

[1]Cows from selection lines for high fat content (HF; 38 cows) or low fat content (LF; 48 cows), respectively, but with similar total milk energy production. SH = 50 cows.

[2]Fatty acids and groups of FA are expressed as g/100 g of FA. CLA = conjugated linoleic acid (refers to *cis*-9,*trans*-11 C18:2); PUFA = C18:2 n-6 + C18:3 n-3 + CLA; PI = polyunsaturated index [C18:2 n-6 + (C18:3 n-3 × 2)].

[3]Contrast between the *DGAT1 AA* and *KA* genotypes.

major reason for this difference should be the dilution of individual cow's milk that occurs in the milk tank. Model 2 to 4 unanimously showed that the most significant risk factor for the development of SOF was high milk copper concentration. Also, the content of unsaturated FA, unadjusted for effect of *DGAT1* polymorphism and other fixed effects (model 2), showed a positive relationship with occurrence of SOF. All of the estimated effects of category of fat and their interaction with copper (model 2) turned out to be significant (Table 5). The interaction between unadjusted concentrations of unsaturated FA and copper (model 2; illustrated for PI in Figure 1) showed that the combination of high milk copper content and high unsaturated FA concentration increased the odds ratio to develop SOF dramatically. When only one of these components was present in high concentrations, the odds ratio increased only marginally. Similar results were reported by Timmons et al. (2001). Clausen et al. (2010) found that the oxidation product hexanal markedly increased with the concentration of added copper ($CuSO_4$) to the milk, but only in the oxidation-sensitive cow, whereas the same additions of copper had no effect on the hexanal concentration in the milk of the oxidation-resistant cow. The oxidation-sensitive and -resistant cows could have been individuals with high and low unsaturated FA concentration, respectively. The effect of breed and selection line on incidence of SOF (model 2) was not significant ($P = 0.13$), and we did not observe any effects of breed and selection line on concentration of copper in milk (Juhlin et al., 2010b).

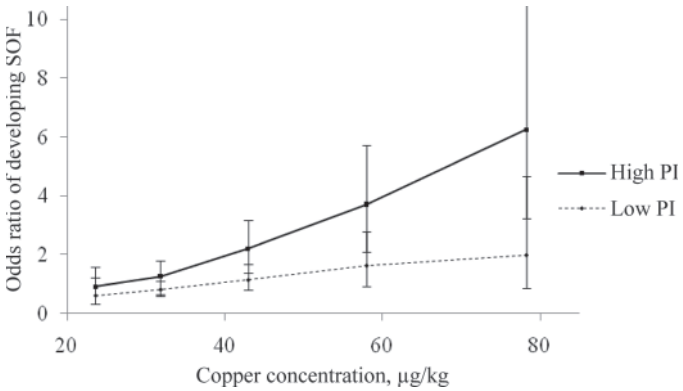

Figure 1. The association of polyunsaturated index (PI) value [FA are expressed as g/100 g of FA; (C18:2 n-6 + (C18:3 n-3 × 2)] and copper content with the risk of milk developing spontaneous oxidized flavor (SOF). Estimates are expressed as odds ratios with 95% confidence intervals. High PI is defined as PI = 5.6; low PI is defined as PI = 2.4.

Model 3, which included *DGAT1* genotype and copper content and their interaction, showed that those parameters are significant in describing the variation in SOF. The *A* allele was associated with a higher risk for SOF, a result that is in line with the effects of the DGAT1 polymorphism on FA composition shown in Table 4, where the *A* allele was associated with long, unsaturated FA (although not always significant at the

Table 5. Significance (*P*-value) of different factors included in various models describing the relationship between spontaneous oxidative off-flavor (SOF) and *DGAT1* genotype, certain (indices of) FA, and copper content in milk from the Swedish Holstein breed (SH) and 2 selection lines[1] of the Swedish Red breed (SR)

		Significance level of the factors used in the model[4]				
Factor[2]	Model[3]	Fat	Fat × logcopper	*DGAT1*	*DGAT1* × logcopper	Logcopper
DGAT1	3			0.0202	0.0241	<0.0001
CLA	2	0.0049	0.0053			<0.0001
AdjCLA+*DGAT1*	4	0.0761	0.0865	0.0140	0.0137	<0.0001
C18:3	2	0.0244	0.0269			0.0002
AdjC18:3+*DGAT1*	4	0.0333	0.0509	0.0005	0.0003	<0.0001
C18:2	2	0.0087	0.0092			0.0002
AdjC18:2+ *DGAT1*	4	0.2626	0.1775	0.0022	0.0014	<0.0001
PUFA	2	0.0047	0.0049			0.0001
AdjPUFA+*DGAT1*	4	0.2724	0.2474	0.0076	0.0071	<0.0001
PI	2	0.0046	0.0048			<0.0001
AdjPI+*DGAT1*	4	0.0110	0.0325	0.0265	0.0110	<0.0001

[1]Cows from selection lines for high fat content (HF; 38 cows) or low fat content (LF; 48 cows), respectively, but with similar total milk energy production. SH = 50 cows.

[2]Fatty acids and groups of FA are expressed as g/100 g of FA. The *DGAT1* genotype is expressed as either *AA* or *AK*. CLA = conjugated linoleic acid (refers to *cis*-9,*trans*-11 C18:2); PUFA = C18:2 n-6 + C18:3 n-3 + CLA; PI = polyunsaturated index [C18:2 n-6 + (C18:3 n-3 × 2)]; the prefix Adj denotes the concentration of (index of) the FA adjusted for *DGAT1* polymorphism and other fixed effects.

[3]See text for explanation of the models.

[4]*P*-values for the factors included in the different models. Fat = the category of fat used in the model; logcopper = the natural logarithm of copper content; fat × logcopper = the interaction term of the category of fat and logcopper; *DGAT1* = *DGAT1* genotype; *DGAT1* × logcopper = the interaction term of *DGAT1* genotype and logcopper.

5% level). When both *DGAT1* genotype and FA content, adjusted for effect of *DGAT1* polymorphism and other fixed effects, were included in the model (4), the effect of adjusted FA content was still significant with regard to C18:3 and PI, but not regarding the other categories of fat (Table 5). This suggests that variation exists in C18:3 n-3 and PI beyond the effect of the *DGAT1* genotype that affects the variation in SOF. This is in line with the observation that *DGAT1* genotype did not significantly affect proportions of C18:3 and PI in milk (Table 4); adjusting the FA concentration for *DGAT1* genotype should consequently not be expected to have a noticeable effect on the effects of these 2 categories of FA on occurrence of SOF. For PUFA, CLA, and C18:2 n-6, using the adjusted values reduced the level of significance below the significance threshold ($P > 0.05$) in comparison with analysis of (unadjusted) FA without *DGAT1* genotype in the model, suggesting that the effect of the different categories of fat on SOF development for those parameters is largely due to *DGAT1* genotype.

CONCLUSIONS

Both copper and PUFA concentrations showed a strong association with occurrence of SOF, where increasing concentrations of both components in milk led to an increased risk for SOF. The interaction between the 2 components implies that SOF will not develop as easily in milk with high copper content unless the substrate is available (i.e., availability of unsaturated FA promote the oxidation process and vice versa). Breed and *DGAT1* genotype had an effect on the contents of long-chain FA. An effect of *DGAT1* genotype on SOF development was observed where the *A* allele, which has previously been associated with a larger proportion of long-chain FA, was related to increased susceptibility of milk to develop SOF.

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