trans-C18:1 Isomers in Cheeses Enriched in Unsaturated Fatty Acids and Manufactured with Different Milk Fat Globule Sizes

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Increasing the knowledge on dietary fat composition, mainly the minor components, will improve the nutritional value of foods and their labeling. In this study, we examined the *trans*-octadecenoic acid (C18:1) composition of Emmental cheeses enriched in unsaturated fatty acids (FA) and manufactured with milks produced by cows selected to produce small and large fat globules. The FA composition of the milks was not significantly ($P > 0.05$) different from the FA composition of the corresponding Emmental cheeses. Increasing the unsaturated FA content of the cheeses using dietary manipulations lead to an increase in the *trans*-C18:1 and changed their isomeric profiles. In milk fat produced with the linseed-enriched diet, the *trans*-10 C18:1 concentration was greater than *trans*-11 C18:1 (vaccenic acid), which is classically the major *trans*-C18:1 in milk fat. The content in *trans*-C18:1 and more particularly in *trans*-10 C18:1 was negatively correlated with the size of fat globules ($r^2 = 0.82$ and 0.87, respectively) and related to milk fat depression. The *trans*-C18:1 content was negatively correlated with the saturated FA (slope $= -0.35$; $r^2 = 0.81$) and positively correlated with the unsaturated (slope $= 0.29$; $r^2 = 0.85$) and monounsaturated (slope $= 0.32$; $r^2 = 0.81$) FA. Focusing on the health-related considerations of fat in food products, further nutritional studies are needed to elucidate the role of *trans*-C18:1 isomers.

KEYWORDS: *trans*-Fatty acid; Emmental cheese; milk fat composition; dairy product

INTRODUCTION

trans-Fatty acids (FAs) in the diet have received higher attention for the last years because of the adverse effects of these isomers with respect to cardiovascular diseases, infant development, diabetes, and inflammation (*1−3*). Dietary *trans*-FAs originate from two major sources: (i) partially hydrogenated vegetable and marine oils obtained by industrial processes that are present in many prepared foods and (ii) ruminant *trans*-FAs formed by biohydrogenation in the rumen of cows, sheeps, and goats that are found in milk and products derived from their meat. Recent studies compared the effects of *trans*-FAs from industrially produced or ruminant (natural) sources on the

markers of cardiovascular disease risk (*4−6*). Increasing the knowledge on the *trans*-FA isomers profile and content in food products is a prerequisite of the estimation of the extent of dietary exposure of young children and adults to *trans*-FAs.

trans-FAs are unsaturated FAs with at least a double bond in the *trans*-configuration, resulting in a more rigid molecule close to a saturated FA. In all dietary sources, the quantitatively most important class of *trans*-FAs is the group of octadecenoic (C18:1) isomers. The *trans*-C18:1 represent about 72% of total *trans*-FAs in milk fat and correspond to an average of 3.7% of total FAs (*7*). The position of the *trans* double bond varies between the Δ6 and the Δ16 carbon atoms of the FA molecule. The Δ11 isomer (vaccenic acid; *trans*-11 C18:1) is the most important in ruminal fat with about 50% of all ruminant *trans*-FA (*7*), whereas the Δ9 isomer (elaidic acid; *trans*-9 C18:1) and the Δ10 isomer (*trans*-10 C18:1) are the most common in partially hydrogenated fats (*8*).

As compared with other natural fats, milk fat has a high level of saturated FAs (65−70%) and a low content in unsaturated FAs (~30%; mainly oleic acid) (*9*). Population studies have long established strong relationships between the saturated FAs

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in the diet and the incidence of coronary heart diseases (*10*), which is a leading cause of death in most industrialized countries. Prospective studies have shown polyunsaturated FAs to be negatively associated with coronary heart disease mortality (*11*), particularly polyunsaturated FAs such as C18:2 (n-6) and C18:3 (n-3). These cannot be formed de novo in humans and are essential for health. Therefore, they need to be ingested from the diet. In this context, modification of the FA profile in milk fat to yield lower saturated FA contents and greater (poly)unsaturated FA contents is a major research focus for the dairy industry. These changes in milk fat composition may be performed using three main ways, which are technology (e.g., dry fractionation), genetic selection of the animal, or dietary changes. Feeding of animals appears to be the more rapid and natural way permitting the increase in the unsaturated FA content of milk (*12*). However, the partial biohydrogenation of the polyunsaturated FA ingested in the diet of cows produces *trans*-FA in the rumen, predominantly *trans*-isomeric vaccenic acid (*12*).

Milk fat is widely consumed in food products (cream and cheeses) in which it is present in the form of fat globules covered by their biological membrane, which is rich in phospholipids (*13*). The size of fat globules ranges from about 0.2 to 10 μm with a volume-weighted diameter of around 4 μm. The nutritional and functional properties of milk fat may depend on both its FA composition and the size of fat globules. Recently, it has been shown that the size of natural fat globules, that is, covered by the milk fat globule membrane (MFGM), may affect the sensorial and technological properties of dairy products (*14*). Fat globules with various sizes can be obtained by gravity separation, microfiltration process, and the animal factors. Moreover, authors have shown that the FA composition of fat globules may vary as a function of their size (*15−17*). However, authors did not focus on the *trans*-FA composition of milk fat globules as a function of their size.

In this study, we changed the FA composition of milk fat using cow diet and focused on the *trans*-C18:1 FA composition of Emmental cheeses manufactured with different milks enriched in unsaturated FAs and produced by cows that secrete small or large milk fat globules. The objectives were (i) to determine the FA composition of milk fats modified using cow diet, (ii) to quantify the *trans*-C18:1 isomers in the Emmental cheeses, and (iii) to determine if processing of milk to manufacture Emmental cheese alters the content and profile of ruminant *trans*-C18:1 positional isomers.

MATERIALS AND METHODS

Animals and Diets for the Production of Cheese Milks. Eighteen Holstein cows with a lactation stage of 105 ± 21 days were selected in the experimental herd of Méjussaume (Le Rheu, France) and were divided into three groups of six cows, corresponding to three different diets. In each group, three cows were selected to produce small milk fat globules (SFG; 3.6 ± 0.1 μm). The other three cows of each group produced large milk fat globules (LFG; 4.3 ± 0.1 μm). The fat content was 26.2 ± 5.6 g/kg in SFG milks vs 36.1 ± 5.7 g/kg in LFG milks. The daily milk yield was 34.5 ± 5.6 kg for SFG cows vs 33.4 ± 5.4 kg for LFG cows. Thus, different sizes of fat globules were obtained naturally using the selection of dairy cows and were based on the amount of fat secreted in the milks. The experimental period was comprised of 6 weeks: The cows were first adapted to the experimental diets for 3 weeks, and the milks were collected for the 3 following weeks. The animals were fed ad libitum. In the first diet named "maize silage", cows were assigned to a maize silage-based diet (70% of the diet) plus a concentrate mixture of cereals (12%) and soybean meal (16.5%). In the experimental diet named "linseed", the maize silage-based diet (70%) was supplemented with the concentrate mixture of

cereals (6.8%), soybean meal (15.5%), and extruded linseed (6.2% of the diet). In the third diet named "rapeseed", the maize silage-based diet (70%) was supplemented with the concentrate mixture of cereals (6.3%), soybean meal (16%), and extruded rapeseed (6.2% of the diet). For the maize silage diet, the fat content was 35.1 g/kg dry matter, whereas the fat content was 49.6 and 50.7 g/kg dry matter for the linseed and rapeseed diets, respectively. Thus, the linseed and rapeseed diets provided the same amount of fat, that is, 4.6% of dry matter. Diets were calculated to be as isoenergetic as possible. For each of the six experimental treatments (combination of diet and milk fat globule size), the milk was collected during one morning milking and the following evening milking and pooled.

Emmental Cheeses. The experimental milks were standardized in fat (3.25%) and protein (3.5%) content, heat treated at 63 °C for 60 s in a plate exchanger, and stored overnight at 4 °C. The next day, small scale (1/100) Emmental cheeses were manufactured in cylindrical, jacketed, stainless steel 10 L vats (Chalon-Mégard, F-01 Montréal la Cluse). The warmed milk (32 °C) was inoculated by *Propionibacterium freudenreichii* (0.01 g/kg, PAL-ITG P9, Laboratoires Standa, F-14 Caen) and bulk starter cultures: 0.14% (vol/wt) *Streptococcus thermophilus* PAL-ITG ST82/87 (Laboratoires Standa, F-14 Caen) and 0.03% (vol/wt) *Lactobacillus helveticus/Lactobacillus delbrueckii* ssp. *lactis* PAL-ITG LH56/LL57. The milk was ripened at 32 °C for 60 min and coagulated by the addition of calf rennet. When the proper firmness of the coagulum was reached (measured by Gelograph, Gel-instrument, CH-Thalwill), the gel was cut into pieces of 4 mm mean diameter. The whey−curd mixture was then cooked at 53.5 °C for 33 min and stirred out. The durations of the foreworking (0−20 min) and the stirring out (30−75 min) were adapted to adjust the moisture contents of the cheeses. The curd was then molded in 12 cm diameter molds. The pressing step (10 kPa for 6 h) and the acidification step (14 h) were conducted at 48 and 36 °C, respectively. The cheeses were then cooled and salted in a NaCl-saturated brine at 7 °C for 5 h. The cheeses (800 g) were wrapped under vacuum in BK1L-ripening bags (Cryovac, F-28 Epernon) and ripened at 12 °C for 21 days and then at 24 °C for 21 days.

Fat Globule Size Measurements. The fat globule size distribution in standardized milks was determined by laser light scattering using a Mastersizer 2000 (Malvern Instruments, Malvern, United Kingdom), equipped with a He/Ne laser (λ = 633 nm) and an electroluminescent diode (λ = 466 nm). The refractive index of milk fat was taken to be 1.460 at 466 nm and 1.458 at 633 nm. The fat globule size distribution was measured after dispersion of standardized milk in 100 mL of distilled water, and 1 mL of 35 mM EDTA/NaOH, pH 7.0, buffer (>98%, disodium salt, 2H$_2$O, Prolabo, France) was added to disrupt the casein micelles. All analyses were performed in triplicate. From the size distribution, the average volume-weighted diameter, $d_{43} = \Sigma N_i d_i^4/\Sigma N_i d_i^3$ (where N_i is the number of fat globules in a size class of diameter d_i), was calculated by the instrument software.

FA Analysis. *Extraction of Fat.* Anhydrous milk fat (AMF) was extracted from the standardized cheese milks according to the following procedure: A 0.8 mL amount of isopropanol (Carlo Erba Reagent, Val de Rueil, France) was added to 10 g of milk, the mixture was vortexed, and then, 1.2 mL of hexane (Carlo Erba Reagenti, Val de Rueil, France) was added. The resulting mixture was vortexed again and centrifuged 5 min at 1000 rpm, 20 °C (Heraeus Cryofuge 7000). The upper organic phase was separated and added to a second organic phase obtained by a second extraction of the lower phase using 1.2 mL of hexane. The pooled fractions were solvent evaporated under nitrogen. For the extraction of fat from the cheeses, pieces of Emmental cheeses were grated and mixed with sand (Sable de Fontainebleau, Grosseron, France) and anhydrous sodium sulfate (Merck, Nogent-sur-Marne, France). AMF was extracted from Emmental cheeses according to Briard et al. (*15*).

Analysis of Total FAs. To obtain total FAs in the cheese milks and the cheeses, after extraction of fat and before gas chromatography, each sample was esterified twice: The first one was butylated to preserve volatile short chain FAs. The second one was methylated with sodium metoxyde to avoid isomerization of long chain unsaturated FAs as described by Briard et al. (*15*). FA esters, methyl and butyl, were analyzed by gas chromatography (Varian model 3800, USA) using a

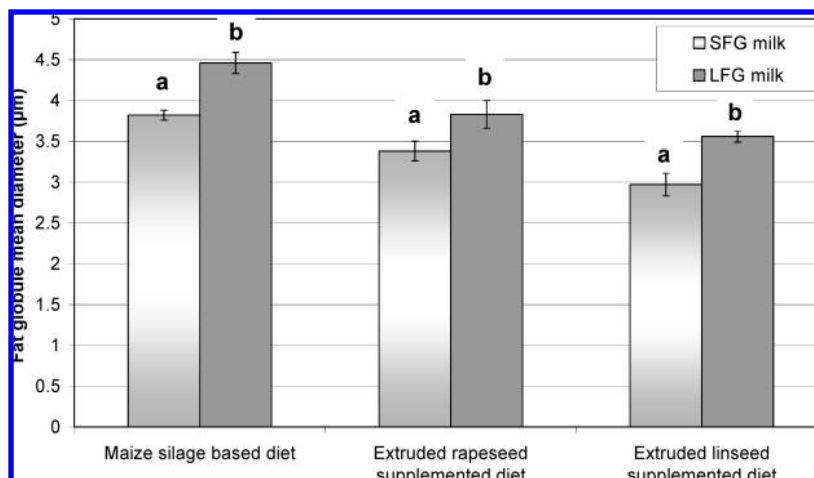**9376** *J. Agric. Food Chem.,* Vol. 56, No. 20, 2008

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Figure 1. Volume-weighted diameter of fat globules in the standardized milks used for the manufacture of Emmental cheeses. The different letters indicate that the diameters are significantly different according to the LSD test ($\alpha < 0.05$).

BPX-70 column (bonded phase, 50 m, i.d. 0.32 mm, film thickness 0.25 μm, SGE, Milton Keynes, United Kingdom) and equipped with a flame ionization detector and a programmed temperature injector. Experimental conditions were as in Briard et al. (*15*).

Butyl esters with C numbers up to C16 and methyl esters from C18 were taken into account. The total FAs profile for butyl esters was thus calculated as follows:

$$\text{final \% FA} = (\text{\% FA-butyl ester}) \times (\text{\% C18-methyl ester}) / (\text{\% C18-butyl ester})$$

Analysis of trans-C18:1 FA Isomers. The analytical procedure was adapted from Juaneda (*18*). FA methyl esters (FAME) prepared with sodium metoxyde were diluted in acetone to a final concentration of 2 mg of FAME in 100 μL of acetone. Two columns (Kromasil-C_{18}, 5 μm, 250 mm × 10 mm i.d., ThermoHypersil, Les Ulis, France) were mounted in series on a Varian 9010 solvent pump (Varian, Les Ulis, France) equipped with a Valco injector fitted with a 100 μL loop. The detector was a differential refractometer (Shimadzu model RID-10A, Shimadzu France, Champs/Marne, France). Acetonitrile was used as a mobile phase at a flow rate of 4 mL/min. The fractions containing the *trans*-C18:1 isomers were collected and injected on a gas chromatograph HP5890 series II (Hewlett-Packard, CA) equipped with a 100 m capillary column coated with 100% cyanopropyldimethyl-polysiloxane (CP-Sil 88, 100 m × 0.25 μm, film thickness 0.2 μm, Chrompack, The Netherlands). The carrier gas was hydrogen. A flame ionization detector was used at 280 °C. The oven temperature was programmed as follows: The initial temperature was 60 °C for 1 min followed by an increase to 160 °C at a rate of 20 °C min^{-1}. The temperature was maintained at 160 °C for 40 min.

Statistical Analysis. Analyses of variance (ANOVA) were performed using the General Linear Model procedure of Statgraphics Plus version 5 (Statistical Graphic Corp., Englewood Cliffs, NJ) to determine the effect of the size of fat globules and the diet of the cows on the FA composition of the Emmental cheeses and cheese milks. Differences between the treatment means were compared at the 5% level of significance using the Fisher's least significance difference (LSD) test.

RESULTS AND DISCUSSION

Fat Globule Size in the Cheese Milks. The size distribution of fat globules was determined in the standardized milks used to manufacture the Emmental cheeses. **Figure 1** shows the mean volume-weighted diameters of fat globules calculated from the size distributions. Whatever the diet, that is, maize silage, linseed, or rapeseed, the diameter of fat globules in the milks was significantly ($P < 0.001$) different, according to the selection of the cows for the production of SFG and LFG (**Figure 1**). Moreover, feeding of dairy cows significantly ($P < 0.001$) affected the size of fat globules in the cheese milks,

as compared to the initial selection of the cows for the production of SFG or LFG (see the Materials and Methods). Cheese milks from cows fed the maize silage-based diet had larger fat globule sizes (LFG, 4.46 ± 0.13 μm; SFG, 3.82 ± 0.07 μm) than rapeseed-enriched diet (LFG, 3.83 ± 0.17 μm; SFG, 3.38 ± 0.12 μm). Cheese milks from cows fed the linseed-enriched diet had the lowest size of fat globules (LFG, 3.56 ± 0.06 μm; SFG, 2.97 ± 0.14 μm) (**Figure 1**). The decrease in fat globule size was related to a decrease in fat production for the experimental diets as compared to the maize silage-based diet (-108 g/day for rapeseed; -270 g/day for linseed). These results are in accordance with Wiking et al. (*16*) who reported that the size of milk fat globules is positively correlated with the diurnal fat production. The possible explanation for the formation of larger fat globules when the synthesis of milk fat increases is the limitation in the production of the MFGM when fat globules are enveloped during their secretion from the epithelial cells of the mammary gland (*16*).

FA Composition of Emmental Cheeses. The chemical composition of the Emmental cheeses was within specified limits for hard type cheeses (*19*). The FA compositions of the Emmental cheeses manufactured with the milks selected for having different milk fat globule sizes, that is, SFG and LFG, and produced with different cow diets are presented **Table 1**. Both cow diet and fat globule size affected the FA composition of the Emmental cheeses.

The saturated FA content in the Emmental cheeses manufactured with the maize silage based diet was 75.8 ± 1.2%, without significant differences due to the size of fat globules. The supplementation of the maize silage-based diet with extruded rapeseed decreased the content in saturated FA to 69.2 ± 2.4%, with 67.4 ± 1.3% for SFG and 71.0 ± 1.8% for LFG. The supplementation of the diet with extruded linseed decreased the content in saturated FA to 66.2 ± 1.7%, without significant differences due to the size of fat globules. As compared to the maize silage-based diet, the linseed-supplemented diet decreased the proportion of FA from C4:0 to C16:0, whereas the rapeseed-supplemented diet decreased the FA from C8:0 to C16:0 and increased the content in C4:0 for SFG (**Table 1**). These FA are those which are mostly synthetised de novo (*12*). Palmitic acid, which is the most abundant FA in milk fat, was significantly ($P < 0.001$) higher in the maize silage-based diet, 36.6 ± 1.3%, as compared to the rapeseed- and linseed-supplemented diets, 29.3 ± 1.4 and 29.2 ± 1.2%, respectively. The content in C16:0 was not significantly different between SFG and LFG (**Table**

trans-Fatty Acids in Hard Type Cheeses

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Table 1. FA Composition (% wt) of Emmental Cheeses Manufactured with Milks Produced by Cows Selected for the Size of Milk Fat Globules (SFG and LFG) and Fed with Three Different Diets: Maize Silage-Based Diet, Rapeseed-Supplemented Diet, and Linseed-Supplemented Diet

	maize silage		rapeseed		linseed		stats[a]		
	SFG	LFG	SFG	LFG	SFG	LFG	diet	size	d × s
C4:0	4.17 ± 0.04	4.18 ± 0.14	4.59 ± 0.07	4.30 ± 0.20	3.83 ± 0.07	3.98 ± 0.14	***	NS	*
C6:0	2.72 ± 0.05	2.85 ± 0.06	2.65 ± 0.08	2.75 ± 0.10	2.30 ± 0.05	2.44 ± 0.05	***	**	NS
C8:0	1.63 ± 0.00	1.80 ± 0.03	1.48 ± 0.07	1.66 ± 0.12	1.39 ± 0.02	1.06 ± 0.50	**	NS	*
C10:0	3.89 ± 0.02	4.08 ± 0.11	3.22 ± 0.17	3.73 ± 0.30	3.33 ± 0.14	2.76 ± 0.65	***	NS	**
C12:0	4.47 ± 0.24	4.57 ± 0.22	3.46 ± 0.13	4.03 ± 0.32	4.10 ± 0.29	3.67 ± 0.12	***	NS	**
C14:0	14.03 ± 0.26	14.08 ± 0.30	13.68 ± 0.22	13.01 ± 0.41	13.89 ± 0.82	13.11 ± 0.48	*	NS	NS
C16:0	36.85 ± 1.94	36.36 ± 0.77	28.55 ± 0.39	30.04 ± 1.37	29.6 ± 1.40	28.52 ± 0.52	***	NS	NS
C18:0	7.50 ± 0.28	7.97 ± 0.40	9.55 ± 0.66	11.27 ± 1.09	7.76 ± 0.30	10.42 ± 0.12	***	***	**
Σ *trans*-C18:1	3.69 ± 0.12	3.05 ± 0.10	5.84 ± 0.18	4.37 ± 0.08	7.53 ± 0.24	6.84 ± 0.11	***	***	**
C18:1 9c (n-9; ω9)	15.08 ± 0.76	15.91 ± 0.71	20.31 ± 1.31	18.70 ± 1.39	18.48 ± 2.16	18.89 ± 0.26	***	NS	NS
C18:1 (n-7)	0.83 ± 0.69	0.41 ± 0.01	1.12 ± 0.89	1.10 ± 0.86	0.73 ± 0.05	0.97 ± 0.13	NS	NS	NS
C18:1 12c	0.27 ± 0.04	0.29 ± 0.02	0.18 ± 0.01	0.16 ± 0.01	0.20 ± 0.04	0.27 ± 0.01	***	NS	NS
C18:1 13c	0.05 ± 0.00	0.05 ± 0.01	0.08 ± 0.01	0.05 ± 0.01	0.12 ± 0.01	0.08 ± 0.00	***	***	**
C18:1 14c	0.32 ± 0.02	0.32 ± 0.02	0.44 ± 0.02	0.36 ± 0.05	0.66 ± 0.02	0.69 ± 0.01	***	*	**
C18:1 D17	0.12 ± 0.01	0.11 ± 0.01	0.18 ± 0.01	0.14 ± 0.02	0.32 ± 0.01	0.37 ± 0.00	***	*	**
C18:2 (Σ)	0.75 ± 0.05	0.76 ± 0.04	1.04 ± 0.11	0.83 ± 0.15	1.76 ± 0.07	1.73 ± 0.01	***	*	*
C18:2 (n-6; ω6)	2.03 ± 0.08	1.95 ± 0.03	2.00 ± 0.06	1.90 ± 0.10	2.12 ± 0.22	2.29 ± 0.04	*	NS	NS
C20:0	0.11 ± 0.00	0.12 ± 0.00	0.14 ± 0.02	0.16 ± 0.02	0.10 ± 0.01	0.13 ± 0.00	***	**	NS
C20:1 (n-11)	0.10 ± 0.01	0.11 ± 0.01	0.14 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.12 ± 0.01	**	NS	NS
C20:1 (n-9)	0.04 ± 0.00	0.04 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.05 ± 0.00	***	NS	NS
C18:3 (n-3; ω3)	0.21 ± 0.01	0.22 ± 0.01	0.29 ± 0.02	0.29 ± 0.02	0.68 ± 0.08	0.72 ± 0.02	***	NS	NS
C18:2 9c 11t (CLA)	0.85 ± 0.04	0.54 ± 0.03	0.80 ± 0.06	0.73 ± 0.07	0.79 ± 0.07	0.78 ± 0.01	**	***	*
C22:0	0.03 ± 0.00	0.06 ± 0.02	0.05 ± 0.01	0.08 ± 0.04	0.04 ± 0.01	0.05 ± 0.01	**	NS	NS
C20:4 (n-6)	0.16 ± 0.01	0.18 ± 0.03	0.12 ± 0.01	0.13 ± 0.01	0.12 ± 0.01	0.13 ± 0.01	***	**	***
Σ saturated FA	75.5 ± 1.7	76.1 ± 0.9	67.4 ± 1.3	71.0 ± 1.8	66.3 ± 2.4	66.2 ± 0.3	***	NS	NS
Σ unsaturated FA	24.5 ± 2.1	23.9 ± 0.9	32.6 ± 1.0	29.0 ± 1.0	33.7 ± 2.4	33.9 ± 0.4	***	NS	NS
Σ monounsaturated FA	20.5 ± 1.4	20.3 ± 0.9	28.4 ± 1.2	25.1 ± 1.7	28.2 ± 2.0	28.3 ± 0.3	***	NS	NS
Σ polyunsaturated FA	4.0 ± 0.1	3.6 ± 0.1	4.3 ± 0.2	3.9 ± 0.2	5.5 ± 0.4	5.6 ± 0.1	***	NS	NS
Σ *trans*-C18:1 + C18:2 9c 11t (CLA)	4.5 ± 0.1	3.6 ± 0.1	6.7 ± 0.1	5.1 ± 0.1	8.3 ± 0.2	7.6 ± 0.1	***	***	**
Σ [*trans*-C18:1 + C18:2 9c 11t]/unsaturated FA (%)	18.6 ± 1.4	15.0 ± 0.3	20.4 ± 0.6	17.7 ± 1.2	24.8 ± 2.1	22.5 ± 0.1	***	***	NS
Σ *trans*-C18:1/C18:1 (%)	18.2 ± 1.7	15.1 ± 0.2	20.8 ± 0.6	17.6 ± 1.3	27.0 ± 2.5	24.3 ± 0.2	***	**	NS
Σ *trans*-C18:1/unsaturated FA (%)	15.1 ± 1.3	12.7 ± 0.2	17.9 ± 0.4	15.1 ± 1.0	22.5 ± 2.1	20.2 ± 0.1	***	**	NS

[a] Analysis of variance; probability of F test: $P < 0.001$ (***), $P < 0.01$ (**), and $P < 0.05$ (*); NS, nonsignificant at $P > 0.05$.

1). The content in stearic acid was significantly ($P < 0.001$) different between the three diets, whatever the size of fat globules: 7.7 ± 0.4% for the maize silage-based diet, 10.4 ± 1.1% for the rapeseed-supplemented diet, and 8.8 ± 1.5% for the linseed-supplemented diet. The content of C18:0 in milk originates both from its concentration in cow diet and from the biohydrogenation of the unsaturated FA with 18 atoms of carbon in the rumen (*12*). As the content in stearic acid was significantly ($P < 0.001$) different between SFG and LFG (**Table 1**), the relationship between the volume-weighted diameter of milk fat globules in milks and the stearic acid content in Emmental cheeses was further examined. **Figure 2** shows the positive linear correlation ($r^2 = 0.96$) between the stearic acid content of Emmental cheeses and the size of fat globules, which was observed for the rapeseed- and linseed-supplemented diets. This result is in accordance with Wilking et al. (*16*), who reported a linear correlation with $r^2 = 0.20$.

The supplementation of the diet with the seeds rich in lipids increased the unsaturated FA content from 24.2 ± 1.2% for the maize silage-based diet, to 30.8 ± 2.4% with rapeseed and to 33.8 ± 1.7% with linseed (**Table 1**). The content in monounsaturated FA was 20.4 ± 1.0% for the maize silage-based diet. The supplementation with rapeseed and linseed increased the level to 26.7 ± 2.2 and 28.2 ± 1.4%, respectively. The oleic acid increased from 15.5 ± 0.8% with the maize silage diet to 18.6 ± 1.5% with linseed and 19.5 ± 1.4% with rapeseed. The content in *trans*-C18:1 changed as a function of cow diet and fat globule size (**Table 1**). The amount of the isomers of *trans*-C18:1 as a function of cow diet and fat globule size is further detailed above. Whatever the size of fat globules, the *trans*-C18:1 corresponded to 13.9 ± 1.5% of the unsaturated
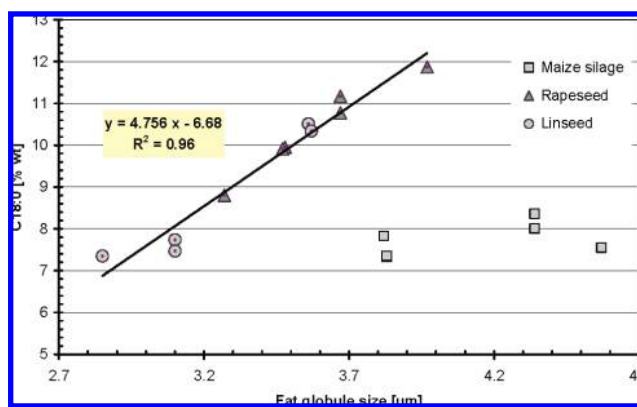

Figure 2. Relationship between the stearic acid content (C18:0) in Emmental cheeses and the volume-weighted diameter of fat globules in the standardized cheese milks for the three diets indicated in the figure.

FA for the maize silage diet, 16.5 ± 1.7% for the rapeseed-enriched diet, and 21.5 ± 2.0% for the linseed-enriched diet. The *trans*-C18:1/unsaturated FA ratio was significantly ($P < 0.01$) different as a function of the size of fat globules (**Table 1**). *trans*-FAs are a minor dietary component, but the foods that contain these FAs affect the balance between the "soft" (*cis*-monounsaturated FA + polyunsaturated FA) and "hard" (saturated FA + *trans*-FA) fats in the diet, which is 2/3 and 1/3, respectively, according to recommended intakes (*20*). **Figure 3** shows the correlation between the content in *trans*-C18:1 and the saturated and unsaturated FA in the Emmental cheeses. The decrease in the saturated FA content of milk fat obtained using
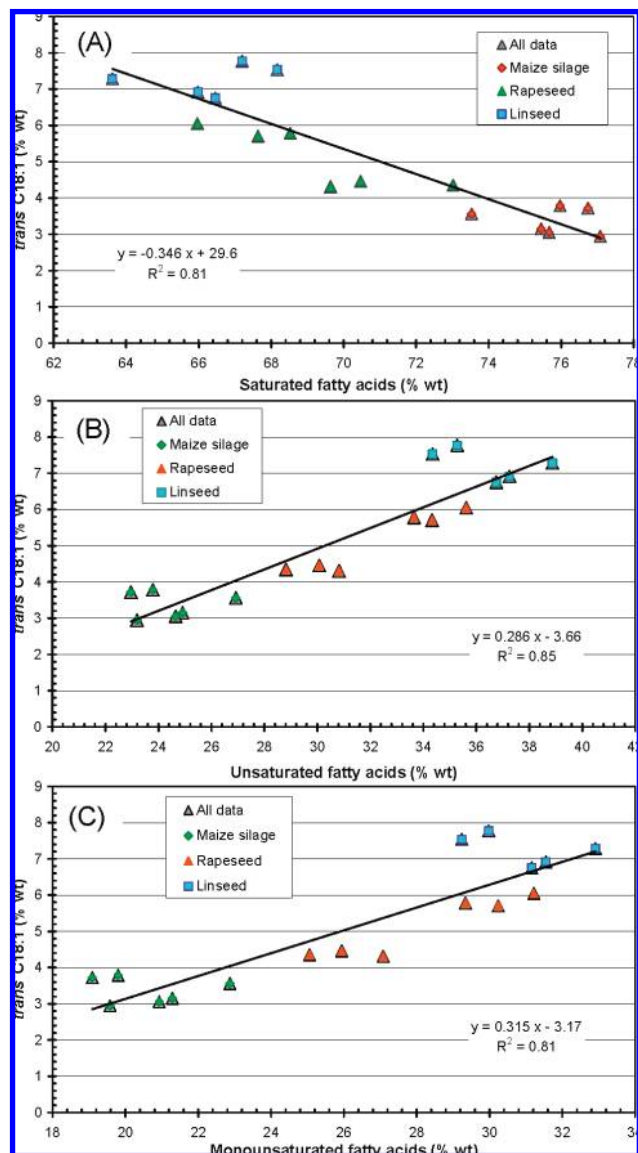
Figure 3. Relationships between the *trans*-C18:1 content in Emmental cheeses and (**A**) the saturated FAs, (**B**) the unsaturated FAs, and (**C**) the monounsaturated FAs.

Figure 4. Profile of *trans*-C18:1 isomers in Emmental cheeses manufactured with milks originating from different diets of cows, (**A**) maize silage, (**B**) rapeseed, and (**C**) linseed, and characterized by SFG or LFG.

cow diet was linearly correlated with the increase in *trans*-C18:1 (slope $= -0.35$; $R^2 = 0.81$; **Figure 3A**). The increase in unsaturated and monounsaturated FA in milk fat is linearly and positively correlated with the content in *trans*-C18:1 (**Figure 3B,C**).

The polyunsaturated FA increased from $3.8 \pm 0.2\%$ with the maize silage-based diet to $4.1 \pm 0.3\%$ with rapeseed- and $5.5 \pm 0.3\%$ with linseed-supplemented diets (**Table 1**). As compared with the other diets, the linseed-supplemented diet significantly ($P < 0.05$) increased the linoleic acid content to $2.2 \pm 0.2\%$. The linolenic acid significantly ($P < 0.001$) increased from $0.21 \pm 0.01\%$ with the maize silage diet to $0.29 \pm 0.02\%$ with rapeseed and $0.70 \pm 0.07\%$ with linseed. The content in the *cis*-9, *trans*-11 C18:2 (rumenic acid, main CLA) increased from $0.68 \pm 0.15\%$ in the maize silage diet to $0.77 \pm 0.07\%$ with rapeseed and $0.78 \pm 0.05\%$ with linseed, whatever the size of fat globules. The sum of *trans*-C18:1 and *cis*-9, *trans*-11 C18:2 (main CLA) corresponded to $4.1 \pm 0.5\%$ in the maize silage-based diet, $5.9 \pm 0.9\%$ in the rapeseed-supplemented diet with significantly ($P < 0.001$) higher *trans*-FA in SFG (**Table 1**), and $8.0 \pm 0.4\%$ in the linseed-
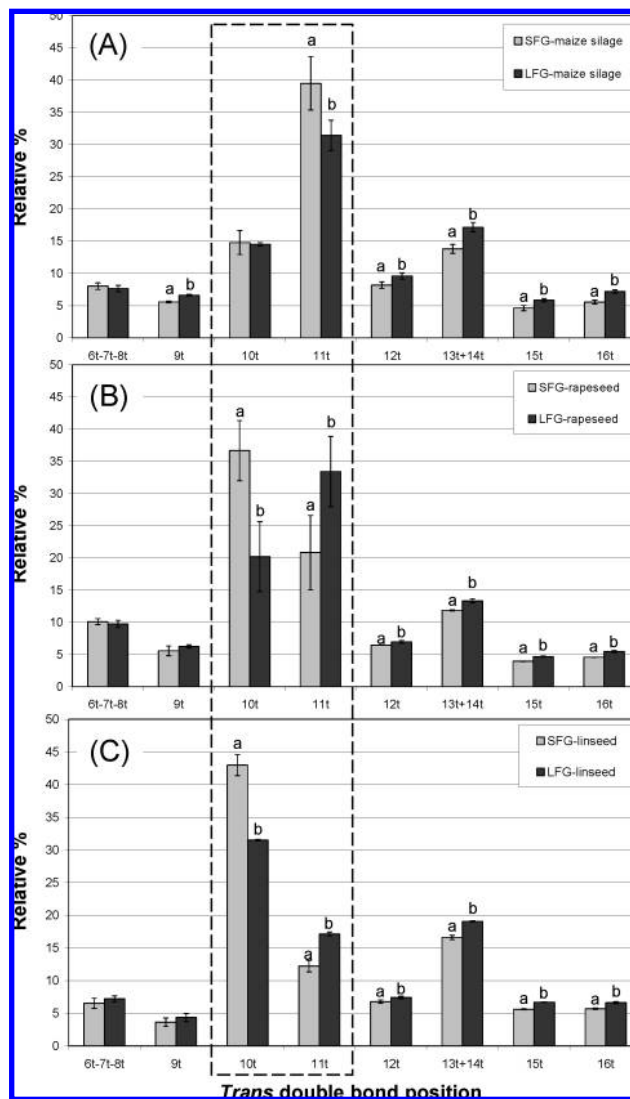
supplemented diet. These total *trans*-FA corresponded to 16.8 ± 2.2, 19.0 ± 1.7, and $23.9 \pm 2.0\%$ of the unsaturated FA for the maize silage diet, rapeseed-enriched diet, and linseed-enriched diet, respectively.

Isomers of *trans*-C18:1 in Emmental Cheeses. The relative profiles of *trans*-C18:1 isomers that were determined in Emmental cheeses for the three diets and for SFG and LFG are presented **Figure 4**. For the maize silage-based diet, the *trans*-11 isomer (vaccenic acid) was the main *trans*-C18:1 with $35.4 \pm 5.4\%$, whatever the size of fat globules (**Figure 4A**). The vaccenic acid was significantly ($P < 0.05$) higher in SFG (39.5 ± 4.2 vs $31.4 \pm 2.4\%$ in LFG). The profile obtained with the maize silage-based diet is classical considering the relative proportions of the *trans*-C18:1 isomers in milk (*7*). For the diet supplemented with rapeseed, a significant ($P < 0.05$) effect of the size of fat globules was observed (**Figure 4B**). For SFG, the main *trans*-C18:1 isomer was the *trans*-10, which represented $36.7 \pm 4.7\%$ of total *trans*-C18:1, whereas the *trans*-11 corresponded to $20.8 \pm 5.8\%$. For LFG, the vaccenic acid was the main *trans*-C18:1 isomer with $33.4 \pm 5.5\%$, and the *trans*-10 represented $20.2 \pm 5.5\%$ of the *trans*-C18:1 isomers. Thus, for the rapeseed-supplemented diet, the LFG showed a classical profile of *trans*-C18:1, while the SFG had an atypical profile

trans-Fatty Acids in Hard Type Cheeses

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Table 2. Amount of Individual *trans*-Octadecenoic Acids in Emmental Cheeses (g per 100 g Fat; Mean ± Standard Deviation), Produced with Three Different Diets (Maize Silage-Based Diet, Rapeseed-Supplemented Diet, and Linseed-Supplemented Diet) and Different Fat Globule Sizes (SFG and LFG) Obtained with Cow Selection

	maize silage[a]		rapeseed[a]		linseed[a]		stat[b]		
trans-C18:1 (g/100 g fat)	SFG	LFG	SFG	LFG	SFG	LFG	diet	size	d × s
trans-6-*trans*-7-*trans*-8	0.30 ± 0.06	0.26 ± 0.03	0.78 a ± 0.04	0.51 b ± 0.05	0.65 ± 0.06	0.62 ± 0.10	***	**	**
trans-9	0.21 ± 0.03	0.22 ± 0.01	0.43 a ± 0.05	0.33 b ± 0.04	0.36 ± 0.07	0.38 ± 0.07	***	NS	NS
trans-10	0.56 ± 0.15	0.49 ± 0.03	2.84 a ± 0.32	1.05 b ± 0.16	4.31 a ± 0.25	2.71 b ± 0.42	***	***	***
trans-11	1.48 a ± 0.06	1.05 b ± 0.03	1.62 ± 0.47	1.80 ± 0.57	1.22 ± 0.08	1.48 ± 0.24	**	NS	NS
trans-12	0.31 ± 0.06	0.32 ± 0.03	0.50 a ± 0.01	0.37 b ± 0.06	0.68 ± 0.02	0.65 ± 0.11	**	NS	NS
trans-13-*trans*-14	0.52 ± 0.1	0.58 ± 0.05	0.92 a ± 0.02	0.71 b ± 0.11	1.66 ± 0.03	1.67 ± 0.29	***	NS	NS
trans-15	0.18 ± 0.04	0.20 ± 0.02	0.31 ± 0.01	0.25 ± 0.04	0.56 ± 0.01	0.59 ± 0.10	***	NS	NS
trans-16	0.21 ± 0.043	0.24 ± 0.02	0.35 ± 0.01	0.29 ± 0.05	0.57 ± 0.01	0.59 ± 0.11	***	NS	NS
trans-10/*trans*-11	0.38 ± 0.09	0.46 ± 0.04	1.93 a ± 0.90	0.63 b ± 0.24	3.54 a ± 0.38	1.83 b ± 0.04	***	***	*
∑ *trans*-FA (g/100 g fat)	3.8 ± 0.5	3.4 ± 0.2	7.8 a ± 0.2	5.3 b ± 0.8	10.0 a ± 0. 3	8.7 b ± 0.4	***	***	**

[a] For each diet, values in the same row with different letters were significantly different according to the LSD test (α < 0.05). [b] Analysis of variance; probability of *F* test: *P* < 0.001 (***), *P* < 0.01 (**), and *P* < 0.05 (*); NS, nonsignificant with *P* > 0.05.

of the *trans*-C18:1 isomers. For the diet supplemented with linseed, the *trans*-10 was the main *trans*-C18:1 isomer, whatever the size of fat globules (**Figure 4C**). For SFG, the *trans*-10 corresponded to 43.0 ± 1.6%, whereas the *trans*-11 corresponded to 12.2 ± 0.9%. For LFG, the *trans*-10 corresponded to 31.5 ± 0.1%, whereas the *trans*-11 corresponded to 17.1 ± 0.3%.

In this study, we showed that cow diet can alter the classical isomeric profile of the *trans*-C18:1 in milk fat (**Figure 4**). More particularly, we showed that the *trans*-10 C18:1 was higher than the *trans*-11 C18:1 for the linseed-supplemented diet and for the SFG milk produced with the rapeseed-supplemented diet (**Table 2** and **Figure 4**). Such a higher proportion of the *trans*-10 isomer as compared to the *trans*-11 isomer has been reported by several authors (*21, 22*). The *trans*-C18:1 in bovine milk fat are formed in the forestomach, and their isomeric profile results from the rumen pH and amount of vegetable oil in the diet for partial biohydrogenation of polyunsaturated FA in the rumen. The metabolic activity of the responsible bacteria includes isomerases producing various *trans*-C18:1 positional isomers (*7*). The results of Griinari et al. (*21*) support the hypothesis that both an altered rumen environment and the presence of unsaturated FA in the diet may enhance *trans*-10 C18:1 in milk fat. The *trans*-10 C18:1 isomer could arise via hydrogenation of *trans*-10, *cis*-12 C18:2 (*23*) or via isomerization of *cis*-9 C18:1 (*24*). Loor et al. (*25*) reported that changes in the microbial ecosystem in the rumen and buffering capacity may be linked with a shift in the production of C18:1 isomers with a *trans*-11 to a *trans*-10 double bond. The *trans*-11 C18:1 (vaccenic acid) is to some extent desaturated to *cis*-9, *trans*-11 conjugated linoleic acid (CLA) by the Δ9 desaturase both in the cow and in the human (*26–28*). **Figure 5** shows the relation between the *trans*-11 C18:1 and the *cis*-9, *trans*-11 C18:2 content in the Emmental cheeses. However, this pathway is not available for the *trans*-9 and *trans*-10 isomers. Thus, the shift of the *trans*-11 C18:1 to the *trans*-10 C18:1 may explain the low content of CLA observed in these experiments (**Table 1**).

The relative proportions of the *trans*-10 and *trans*-11 C18:1 isomers were affected by the cow diet and the size of fat globules (**Figure 4**). However, the sum of *trans*-10 and *trans*-11 was not significantly (*P* > 0.05) affected by cow diet: 50.1 ± 5% for maize silage, 55.6 ± 2.3% for rapeseed, and 52.6 ± 3.6% for linseed. Whatever cow diet, the sum of these two isomers was affected by the size of fat globules and was significantly (*P* < 0.01) higher for SFG, 55.7 ± 2.0%, as compared to LFG, 49.5 ± 3.8%.
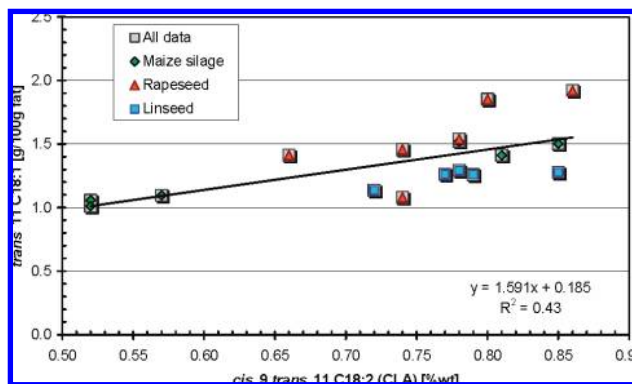

Figure 5. Relationship between the content in vaccenic acid (*trans*-11 C18:1) and the content in rumenic acid (*cis*-9, *trans*-11 C18:1; main conjugated linoleic acid, CLA) in the Emmental cheeses. The diets are indicated in the figure.

The amount of the isomers of *trans*-C18:1 was determined in the Emmental cheeses (**Table 2**). The Emmental cheeses manufactured from the milks produced with the linseed- and the rapeseed-supplemented diets were richer in *trans*-FA than the maize silage-based diet (**Table 2**). Then, the total amount of *trans*-C18:1 in Emmental cheeses (expressed in g per 100 g fat) depends on cow diet. This result is not surprising since the *trans*-FAs are produced by rumen microflora as intermediates in the biohydrogenation of unsaturated FAs until the formation of stearic acid (*12*). Thus, the content of *trans*-FA in milk fat depends on the amount of polyunsaturated FA in the diet. Craig-Schmidt and Holzer (*29*) reported that *trans*-FAs in milk fat are in the range from about 1 to 8%, depending on the season and the region. Our results are in accordance with the latter author (**Table 1**). Moreover, we showed that the supplementation of the maize silage-based diet with extruded linseed resulted in about 10 g of *trans*-FA per 100 g fat (**Table 2**). The amount in *trans*-C18:1 also depends on the size of fat globules for the experimental diets (**Table 2**). Quantifying the content in *trans*-FA in food products is of primary importance since dietary intakes and particularly the daily maternal diet clearly influence the contribution of *trans*-FA to the FA composition in human breast milk. Moreover, it has been shown that the consumption of cow's milk fat and dairy products rich in *trans*-FA by pregnant and lactating women may affect the FA composition of breast human milk and the health of newborn infants (*30, 31*).

From a nutritional point of view, the multiplicity of positional isomers of *trans*-FA formed by biohydrogenation may have distinct metabolic and health effects. Thus, particular attention

Table 3. *trans*-Octadecenoic Acid Composition of Cheese Milks (Mean ± Standard Deviation), Produced with Three Different Diets (Maize Silage-Based Diet, Rapeseed-Supplemented Diet, and Linseed-Supplemented Diet) and Different Fat Globule Sizes (SFG and LFG) Obtained with Cow Selection

trans-C18:1 (%)	maize silage[a]		rapeseed[a]		linseed[a]		stat[b]		
	SFG	LFG	SFG	LFG	SFG	LFG	diet	size	d × s
trans-6-*trans*-7-*trans*-8	7.0 ± 1.4	8.1 ± 0.2	9.3 ± 1.8	8.9 ± 1.0	6.5 ± 1.3	7.1 ± 0.2	***	NS	NS
trans-9	6.1 ± 0.5	6.6 ± 0.1	5.4 a ± 0.4	6.2 b ± 0.4	3.1 a ± 0.2	4.4 b ± 0.1	***	**	NS
trans-10	15.1 ± 1.9	14.3 ± 0.1	33.8 a ± 4.2	18.2 b ± 5.7	40.7 a ± 3.4	30.9 b ± 0.4	***	***	**
trans-11	38.9 a ± 7.1	31.7 b ± 2.6	24.2 a ± 4.4	35.5 b ± 6.3	13.2 a ± 2.4	17.1 b ± 0.4	***	NS	**
trans-12	8.3 a ± 0.7	9.3 b ± 0.1	6.6 a ± 0.8	7.3 b ± 0.4	7.3 ± 1.4	7.5 ± 0.2	***	***	NS
trans-13-*trans*-14	14.4 a ± 0.7	16.5 b ± 1.1	12.0 a ± 0.6	13.5 b ± 0.2	17.1 a ± 0.8	19.4 b ± 0.2	***	***	*
trans-15	4.8 a ± 0.5	5.8 b ± 0.6	4.1 a ± 0.3	4.8 b ± 0.2	5.8 a ± 0.2	6.8 b ± 0.1	***	***	NS
trans-16	5.5 a ± 0.4	7.2 b ± 0.7	4.6 a ± 0.3	5.6 b ± 0.1	5.9 a ± 0.3	6.9 b ± 0.1	***	***	**
trans-10/*trans*-11	0.40 ± 0.15	0.45 ± 0.04	1.40 a ± 0.32	0.53 b ± 0.26	3.08 a ± 0.28	1.81 b ± 0.06	***	***	*
$\sum$ *trans*-FA (g/100 g fat)	3.92 ± 0.26	3.35 ± 0.21	7.86 a ± 0.23	5.38 b ± 0.32	9.85 a ± 0.33	8.82 b ± 0.33	***	***	**

[a] For each diet, values in the same row with different letters were significantly different according to the LSD test ($\alpha < 0.05$). [b] Analysis of variance; probability of F test: $P < 0.001$ (***), $P < 0.01$ (**), and $P < 0.05$ (*); NS, nonsignificant with $P > 0.05$.

should be drawn to the *trans*-C18:1 positional isomers as a risk to health caused by *trans*-FA may be possibly related to certain isomers to a different extent (*7*).

Comparison of the FA Composition in Milks and Emmental Cheeses. Whatever the cow diet and the size of milk fat globules, the FA composition of the cheese milks was not significantly ($P > 0.05$) different from the FA composition of the corresponding Emmental cheeses (results not shown). Moreover, the total content and relative amount of *trans*-C18:1 isomers were not significantly different ($P > 0.05$) between the cheese milks (**Table 3**) and the corresponding Emmental cheeses. These experiments showed that the thermal and mechanical treatments applied during the processing of milk to manufacture Emmental cheeses did not affect the amount and composition profile of FA and, more particularly, of the *trans*-C18:1. Furthermore, the enzymatic and chemical reactions (lipolysis, catabolism of FAs) that occurred during ripening did not affect the content in *trans*-C18:1. Thus, the determination of the *trans*-C18:1 isomer profile and content in the milk reflects their amount in the dairy products. Considering another class of *trans*-FA, Gnädig et al. (*32*) showed that the manufacturing and ripening conditions used in the production of Emmental cheese do not affect the isomer composition and the content of the conjugated linoleic acid (CLA).

Relation between the Content in *trans*-C18:1 and the Size of Fat Globules. Figure 6 shows a negative correlation between the amount of *trans*-C18:1 in the Emmental cheeses and the size of fat globules, for the three diets considered in this study. The largest amount of *trans*-C18:1 corresponded to the smallest fat globules produced by the cows ($r^2 = 0.82$; **Figure 6A**). The sum of the most important isomers of *trans*-C18:1, that is, *trans*-10 and *trans*-11 (53 ± 4% of total *trans*-C18:1), was also negatively correlated with the size of fat globules ($r^2 = 0.84$; **Figure 6B**). The content in *trans*-10 C18:1 was well-correlated with the size of fat globules ($r^2 = 0.87$; **Figure 6C**), whereas no relation was found between the content in *trans*-11 C18:1 and the size of fat globules.

This negative correlation between the content in *trans*-C18:1 and the size of fat globules may result from (i) the mechanisms of synthesis and secretion of fat globules and/or (ii) the composition of the MFGM. Considering the first hypothesis, it is important to discuss the role of *trans*-FAs on milk fat depression and the consequence on fat globule size. Across a substantial number of published studies cited in Loor et al. (*22*), data clearly suggest that an increased percentage of *trans*-10 C18:1 in milk fat is positively correlated with milk fat depression in cows fed high-concentrate diets with or without unsaturated
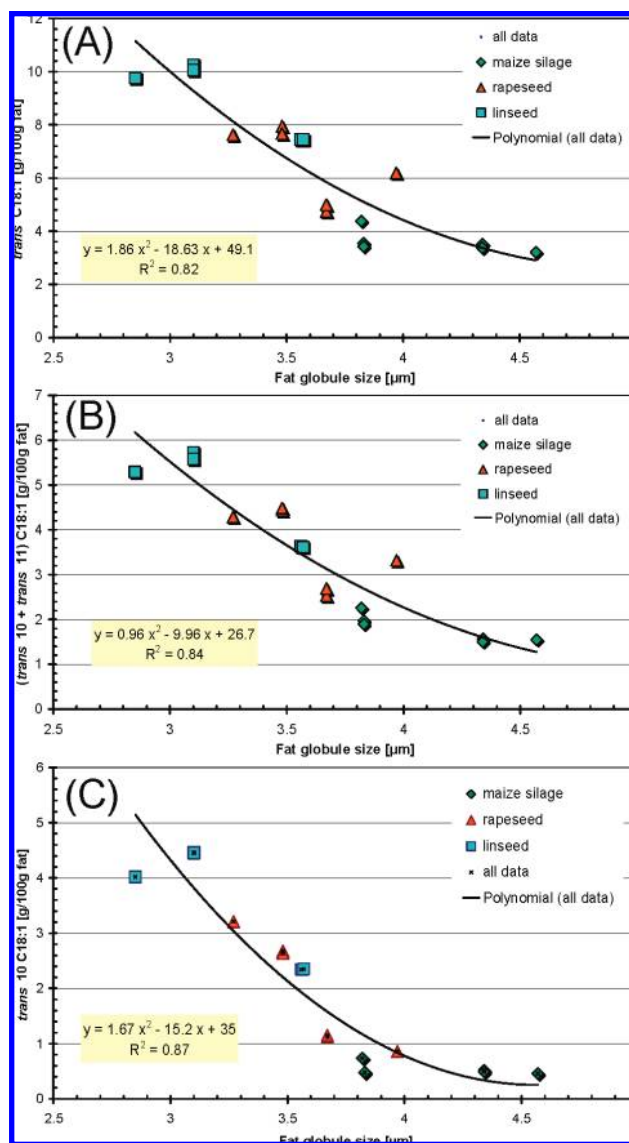

Figure 6. Relationships between the content in *trans*-C18:1 FAs in the Emmental cheeses and the volume-weighted diameter of fat globules in the standardized cheese milks. (**A**) *trans*-C18:1, (**B**) (*trans*-10 + *trans*-11) C18:1, and (**C**) *trans*-10 C18:1.

oils or mixed diets with various levels of fish oil (*21*). This depression of fat in milk may induce a reduction of fat globule size since more MFGM material is susceptible to cover the

triacylglycerol core (*16*). Thus, the linseed- and rapeseed-supplemented diets may have affected the metabolism of lipids in the rumen due to the high content in unsaturated FA and an altered ruminal environment and change the way of synthesis of fat. As a consequence, we observed a higher content of *trans*-10 C18:1 and a decrease in fat globule size associated with milk fat depression. Considering the second hypothesis, the smallest fat globules have a higher interfacial area and then a higher amount of the MFGM for the same amount of total fat. Thus, the phospholipids/triacylglycerols ratio increases as a function of the decrease of the size of fat globules. Further studies are needed to determine the *trans*-C18:1 composition of the MFGM. For the first time to the author's knowledge, the higher amount of *trans*-10 C18:1 was quantified in SFGs and explained with milk fat depression.

As a conclusion, this study showed that dietary polyunsaturated FAs that are active in the rumen affect the FA composition of milk fat in dairy products and more particularly the *trans*-C18:1. The supplementation of the maize silage-based diet with extruded rapeseed and linseed decreased the saturated FA and increased the unsaturated FA. As compared to the rapeseed-enriched diet, the linseed-supplemented diet resulted in a larger amount of polyunsaturated FA and *trans*-C18:1, which is due to the higher content in C18:3 (n-3) in the linseed and to the biohydrogenation and isomerization of the unsaturated FAs, which occur in the rumen. These lipid-rich diets increased the content in *trans*-C18:1 and changed the profile of the isomers. Moreover, this work showed that the size of fat globules depends on fat production, on cow diet, and on metabolic perturbations in the rumen. For the first time, the size of fat globules was related to their content in *trans*-FAs. Furthermore, we showed that the processing of milk to manufacture Emmental cheeses did not alter the *trans*-FA profile and content.

From a nutritional point of view, the remaining question is: Do dietary changes performed to increase the unsaturated FA content in milk fat alter the nutritional quality of bovine lipids, by increasing the *trans*-C18:1 and by changing their isomeric profile? The effects of the isomers of *trans* FAs on blood lipids and the pathological consequences should be one of the elements to be considered in the evaluation of possible strategies for changes in the FA composition of milk fat using dietary manipulations.

ACKNOWLEDGMENT

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