J. Dairy Sci. 90:392–403

Composition of Milk Fat from Cows Selected for Milk Fat Globule Size and Offered Either Fresh Pasture or a Corn Silage-Based Diet

S. Couvreur,[1] C. Hurtaud,[2] P. G. Marnet, P. Faverdin, and J. L. Peyraud
Unité Mixte de Recherches INRA-Agrocampus Rennes Production du Lait, Domaine de la Prise, 35590 Saint-Gilles, France

ABSTRACT

The objective of this study was to examine the synthesis and composition of milk produced by dairy cows that secrete either small milk fat globules (SMFG) or large milk fat globules (LMFG), and to study their response to diets known to alter milk composition. Four groups of 3 multiparous dairy cows were assigned to 2 isoenergetic feeding treatments: a corn silage treatment supplemented with soybean meal, and fresh pasture supplemented with cereal concentrate. The 4 groups comprised 2 groups of 3 dairy cows that produced SMFG (3.44 μm) and 2 groups of 3 dairy cows that produced LMFG (4.53 μm). The SMFG dairy cows produced higher yields of milk, protein, and calcium. Nevertheless, their milk had lower fat and protein contents. Both SMFG and LMFG cows secreted similar amounts of milk fat; therefore, higher globule membrane contents in milk fat were observed in SMFG cows. Higher calcium mineralization of the casein micelles in SMFG cows suggests that it may be possible to improve cheese-making properties even if the lower protein content may lead to lower cheese yields. The SMFG cows secrete milk fat with a higher concentration of monounsaturated fatty acids and a lower concentration of short-chain fatty acids. They also have a higher C18:1/C18:0 ratio than LMFG cows. This suggests that SMFG cows have more significant fatty acid elongation and desaturation. The pasture treatment led to an increase in milk and protein yields because of increased energy intake. It also resulted in lower milk fat yield and fat and protein contents. The pasture treatment led to a decrease in milk fat globule size and, as expected, an increase in monounsaturated and polyunsaturated fatty acid contents. However, it induced a decrease in the protein content, and in calcium mineralization of casein micelles, which suggests that this type of milk would be less suitable for making cheese. This study also shows that there is no correlation between the cows, based on milk fat globule size and diet. These results open up possibilities for improving milk fat quality based on milk fat globule size, and composition. The mechanisms involved in milk fat globule secretion are still to be determined.

Key words: milk fat globule, pasture, fatty acid composition, milk composition

INTRODUCTION

Native milk fat is found in milk in the form of fat globules (**MFG**), the diameter of which ranges from 0.2 to 20 μm; the mean diameter of MFG is around 4 μm (Mulder and Walstra, 1974). Native MFG are essentially droplets of triglycerides surrounded and stabilized in the milk's aqueous phase by a membrane that is derived from the plasma membrane and the contents of the mammary epithelial cell (Mulder and Walstra, 1974; Keenan and Dylewski, 1995). The role played by MFG size in the quality of dairy products has previously been studied through separation by skimming or tangential microfiltration of 2 MFG populations of different size from the same milk (Michalski et al., 2003, 2004; O'Mahony et al., 2005). Small MFG (**SMFG**) milk resulted in more elastic rennet gel (Xiong and Kinsella, 1991; Michalski et al., 2002). Cheeses made with SMFG milk, whether soft or hard cooked, are more flexible and less firm, with less storage potential (Michalski et al., 2003, 2004).

Few studies have attempted to determine the mechanisms of MFG secretion and the factors that can modify MFG size (Heid and Keenan, 2005). Milk fat globule size is correlated with fat content and could be linked to the quantity of milk fat secreted and the concentration of long-chain FA in milk (Wiking et al., 2004). Adding unsaturated fats (canola) to a corn silage diet or replacing corn silage with pasture reduces the mean diameter of MFG by about 0.3 μm (Avramis et al., 2003; Wiking et al., 2003). Furthermore, there is significant variation (up to 1 μm) of the MFG size between individual cows (Mulder and Walstra, 1974). These results open up the possibility of selecting cows according to MFG size to modify milk fat processibility. Nevertheless, as no information exists on milk characteristics

Received April 7, 2006.
Accepted August 25, 2006.
[1]Present address: Groupe ESA, Equipe Enseignement-Recherche en Zootechnie, 55 rue Rabelais, BP30748, 49007 Angers Cedex 01, France.
[2]Corresponding author: Catherine.Hurtaud@rennes.inra.fr

from cows varying in MFG size, the consequences of such a type of selection on milk composition are not known.

The objectives of this study were 1) to identify the differences in milk composition between dairy cows that produced SMFG and those that produced large milk fat globules (**LMFG**), 2) to study the response of these 2 types of dairy cow to diets known to alter milk composition, and 3) to formulate hypotheses, based on the results obtained, on mechanisms of MFG secretion.

MATERIALS AND METHODS

Animals and Diets

The study was based on a crossover design with 12 Holstein cows, at 165 ± 28.3 DIM, divided into 4 groups of 3 cows each. Two groups comprised cows that produced SMFG (volume-weighted diameter of 3.44 ± 0.17 μm), 32.1 ± 4.6 kg/d milk with 3.73% (± 0.76%) fat content, and 3.03% (± 0.16%) protein content. The 2 other groups comprised cows that produced LMFG (volume-weighted diameter of 4.53 μm ± 0.13 μm), 24.3 ± 5.0 kg/d milk with 4.44% (± 0.47%) fat content, and 3.59% (± 0.10%) protein content.

Within each group, cows were assigned to either a corn silage (**CS**) or pasture (**Pt**) treatment in a crossover design from April 1 to May 28, 2004. Each experimental period lasted 4 wk and comprised 21 d of diet transition and 7 d of data collection.

Diets were calculated to be as isoenergetic as possible. Over the 2 wk of data collection, strip-grazing was practiced. Each cow had access to approximately 22 kg of DM of pasture per day (perennial ryegrass and white clover). Given these conditions, cows were expected to eat about 15 kg of DM of pasture per day. Grazing surface per day was calculated using the estimated available DM biomass on the paddock the previous day, determined by cutting with a motorscythe at 5 cm above ground level. Cows grazed on 2 fields sown with a combination of perennial ryegrass and white clover in the fall of 1998. Cows fed on pasture also received 3.3 kg DM of cereal concentrate consisting of corn (29% of DM), barley (29% of DM), fine bran wheat (15.5% of DM), canola meal (11% of DM), $CaCO_3$ (4.6% of DM), beet molasses (4% of DM), vegetable oil (2.5% of DM), magnesium oxide (1.8% of DM), $NaHCO_3$ (1% of DM), and salt (1% of DM), and received supplements of 250 g of 5-18-10 (P-Ca-Mg) minerals (0.6% of DM). The CS treatment was calculated to provide the same amount of energy as the Pt treatment. The CS diet was provided as a TMR consisting of 82.5% forage and 17.5% concentrate. On average, it consisted of 15.5 kg of DM/d of corn silage and 3.3 kg of DM/d of soybean meal per cow, supplemented with 250 g of 5-18-10 (P-Ca-Mg) minerals. Table 1 presents the characteristics of the forages and concentrates used in the experiment. Cows in the CS treatment were housed in cubicle housing systems, and water was available at all times.

Table 1. Composition and nutritional value of forages and concentrates

	Corn silage	Ryegrass and white clover	Soybean	Cereal concentrate
OM, %	95.7	89.9	93.0	86.5
CP, %	5.7	18.0	49.1	14.3
NDF, %	47.5	50.9	14.6	17.8
ADF, %	25.6	23.9	7.6	6.8
Ether extract, %	2.4	ND[1]	3.0	ND
NE_L, Mcal/kg of DM	1.51	1.79	2.02	1.65
PDIE,[2] g/kg of DM	65	102	351	99
PDIN,[3] g/kg of DM	35	113	248	109

[1]ND = Not determined.

[2]PDIE = Protein digested in the small intestine supplied by rumen-undegraded dietary protein and by microbial protein from rumen-fermented organic matter (INRA, 1989).

[3]PDIN = Protein digested in the small intestine supplied by rumen-undegraded dietary protein and by microbial protein from rumen-degraded organic matter (INRA, 1989).

Sampling Schedule and Procedure

Feed. The amounts of corn silage, soybean, and cereal concentrate provided and orts were weighed at each meal. Pasture intake at grazing (Pt treatment) was estimated using the GrazeIn model (Delagarde and O'Donovan, 2005). GrazeIn is a predictive model of pasture intake by grazing dairy cows. Initially, the potential intake (i.e., ad libitum intake) was calculated in a submodel based on the INRA Fill Unit system (INRA, 1989), taking into account cow intake capacity, fill value of herbage, and substitution rate between pasture, roughage, and concentrate supplements. Secondly, at grazing, the relative intake was calculated in a submodel taking into account pasture allowance and pregrazing herbage mass, enabling all the interactions between cows, sward, supplements, and grazing management to be calculated.

Each data collection week, 2 daily samples (50 g) of corn silage, soybean, and cereal concentrate were taken. They were pooled for each period and stored at –20°C. Dry matter (80°C, 48 h), total N by the Dumas method (Association Française de Normalisation, 1985), ash (incineration at 550°C for 5 h), cell wall constituents (NDF and ADF) analyzed sequentially on Fibersac (Ankom Technology Corporation, Fairport, CT) according to Van Soest et al. (1991), and ether extract were measured. Two daily samples (700 g) of fresh pasture were taken by cutting with a motorscythe at 5 cm above ground level during each experimental week. Dry mat-

ter content, total N content, mineral, cell wall constituents (NDF and ADF), and ether extract were determined. Energy and protein values of the feeds were calculated according to INRA (1989). The pasture height was measured with a plate meter on each daily plot.

Milk Yield and Composition. Cows were milked at 0700 and 1800 h throughout the trial. Milk yield was recorded at each milking. Fat and protein contents were individually determined at each milking between d 21 and 27 by infrared analysis (Milkoscan, Foss Electric, Hillerød, Denmark). For each cow, on d 25 of each period, 1-L samples of milk were collected by mixing morning and evening milks in representative proportions. These samples were analyzed for total N (Kjeldahl), true protein N (precipitation at pH 4.6 with TCA, and filtration), casein (precipitation at pH 4.6 with 10% acetic acid and 1 *M* sodium acetate; Rowland, 1938), and total and soluble calcium (by atomic absorption spectrophotometry on milk and milk ultrafiltrate, respectively).

MFG Size. Milk fat globule size was determined by laser light scattering using a Mastersizer 2000 (Malvern Instruments, Malvern, UK) equipped with 2 different wavelengths (He/Ne laser: 633 nm; electroluminescent diode: 466 nm). The obscuration rate was fixed at 10%. Milk samples were measured by diluting with 35 m*M* EDTA/NaOH, pH 7.0, buffer (1:1 vol), to dissociate casein micelles, and then dispersing in a sample unit containing 100 mL of 0.1% (wt/vol) SDS solution in purified water. The absorption coefficients of liquid milk fat used for measurement were 0.5×10^{-5} and 1.7×10^{-5} at 633 and 466 nm, respectively. The refractive indices of MFG in water were 1.458 and 1.460 at 633 and 466 nm, respectively (Michalski et al., 2001). The diameter of the distribution peak (mode), sauter diameter $d_{3,2} = \Sigma(N_i \times d_i^3)/\Sigma(N_i \times d_i^2)$, the volume-weighted diameter $d_{4,3} = \Sigma(N_i \times d_i^4)/\Sigma(N_i \times d_i^3)$, and the specific area $S = 6/(\rho \times d_{3,2})$ were calculated based on the size distribution (in % vol/vol), using Malvern software [where N_i is the number of fat globules in a size class of diameter d_i and ρ is the particle density (0.92 for milk fat); Mulder and Walstra, 1974]. The membrane surface area synthesized daily was calculated by multiplying the specific surface area by the fat production. The volume of membrane synthesized daily was calculated by multiplying the membrane surface area synthesized daily by the thickness of the membrane (10 nm according to Mulder and Walstra, 1974). The proportions of membrane in milk fat and in milk were calculated by dividing the amount of membrane synthesized daily by fat yield and milk yield, respectively.

Milk Fatty Acid Composition. Milk lipids were extracted from 0.5 mL of milk with 0.5 mL of ethanol (96.2°):hydrochloric acid (4:1 vol) and 5 mL of n-hexane. After centrifugation, the supernatant was extracted, and evaporated under nitrogen flux in warm water (<25°C). The extracted lipids were saponified with methanolic sodium hydroxide solution (0.5 *N* NaOH in 100 mL of methanol) at 70°C for 15 min. Fatty acids (**FA**) were methylated with 1 mL of BF3 (20% boron in trifluoride-methanol complex) at 70°C for 10 min. Fatty acid methyl esters were extracted with n-hexane, and analyzed by gas chromatography using a GIRA 1600 chromatograph (GIRA, Morlaas, France) with a split injector (1:10) at 240°C, and a bonded silica capillary column (120 m × 0.25 mm i.d., BPX 7; SGE, Villeneuve-St-Georges, France) with a stationary phase of 70% cyanopropylpolysilphenylene-siloxane (0.25-μm film thickness). Helium was used as the gas vector (10^5 Pa). After initiation, the column temperature program remained constant at 60°C for 7 min, increasing at a rate of 7°C/min to reach 150°C, then increasing at a rate of 0.7°C/min to reach 210°C, and subsequently remaining at 210°C for 10 min. The flame-ionization detector temperature was 260°C. Identification of FA methyl ester peaks was based on retention times obtained for methyl esters prepared from FA standards. Fatty acid profiles were also established based on certified reference materials of anhydrous milk fat (CRM 164 no. 977, Community Bureau of Reference, BCR, Brussels, Belgium). After comparison of measured values and reference values for CRM 164, an error factor was calculated and applied to the measurement of short-chain FA levels (C4:0 to C14:0) in our samples. Fatty acid yield (g/d) was determined by multiplying the percentage of each FA by milk fat yield and by 0.86, a value corresponding approximately to the percentage of FA in triglycerides.

Statistical Analysis

All data were analyzed using the MIXED procedure of SAS (SAS Institute, 1990) using a crossover design. The linear model used is described by the following equation:

$$Y_{ijkl} = \mu + MFG_i + C_{j(i)} + D_k + P_l + D_k \times MFG_i + P_l \times MFG_i + e_{ijkl} + b \times Cov_{ijkl}$$

where Y_{ijkl} = variable studied during the trial; μ = the overall mean of the population; MFG_i = the effect due to MFG size where i equals SMFG or LMFG (tested against the mean squares of cow within MFG group effect); $C_{j(i)}$ = the effect due to cow j characterized by MFG size where i equals SMFG or LMFG; D_k = the effect due to treatment where k equals Pt or CS; P_l = the effect due to period l; $D_k \times MFG_i$ = the interaction between treatment and MFG size; $P_l \times MFG_i$ = the

Table 2. Milk production and composition from cows selected for small milk fat globule (MFG) size (SMFG) or large milk fat globule size (LMFG) and offered either fresh pasture (treatment Pt) or a corn silage-based diet (treatment CS)[1]

	Forage		Globule size				Effects	
	CS	Pt	SMFG	LMFG	SD	SD_{MFG}[2]	Forage	MFG
Yield, kg/d								
Milk	22.7	25.6	27.0	21.3	0.85	1.58	***	***
Fat	0.99	0.96	0.96	1.00	0.039	0.109	†	NS
Protein	0.75	0.82	0.83	0.74	0.037	0.060	***	*
Composition, %								
Fat	4.47	3.81	3.58	4.70	0.216	0.254	***	***
Protein	3.32	3.22	3.08	3.46	0.082	0.118	*	**

[1]The results shown are the average values from 7 d of milking (between d 21 and 27 for each experimental period). No interaction between forage type and MFG cows was observed.

[2]SD_{MFG} = Standard deviation to test the MFG effect.

†$P \leq 0.1$; *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$.

interaction between period and MFG size; e_{ijkl} = error associated with each Y_{ijkl}; $b \times Cov_{ijkl}$ = a covariable based on the average value observed for variable Y (for milk yield, fat and protein contents, fat and protein yield) or for fat yield (for milk FA yield) during a preexperimental week before the beginning of the trial.

The procedure used for the analysis of MFG size descriptors, milk calcium composition and yield, and milk FA composition was the same but without covariance.

RESULTS

For all measurements, no interaction between forage type and MFG cows was observed.

Intake, Milk Production, and Energy and Protein Balances

The grass in the paddock before grazing was high (19.8 ± 2 cm) and large quantities of grass were available in the Pt treatment (22.2 ± 1.6 kg of DM/cow per d above 5 cm).

Estimated pasture intake and actual corn silage and concentrate intake were similar between SMFG and LMFG cows (15.5 kg/d). Estimated NE_L intake and balance were also similar between SMFG and LMFG cows. Estimated pasture intake and measured corn silage and concentrate intakes were similar between the Pt and CS treatments. Estimated NE_L intake and balance were higher with treatment Pt (19.9 vs. 17.2 Mcal/d, and 2.4 vs. –0.4 Mcal/d for NE_L intake and NE_L balance, respectively).

The SMFG cows had higher milk yields than LMFG cows (+5.7 kg/d, $P = 0.009$), lower fat content (–1.12%, $P < 0.001$), and lower protein content (–0.38%, $P = 0.004$; Table 2). Protein yield was greater for SMFG cows (+0.08 kg/d, $P = 0.05$). No difference in fat production between SMFG and LMFG cows was observed. The Pt treatment increased milk production and protein yield (+2.9 and +0.08 kg/d, respectively). The Pt treatment reduced milk fat (–0.66%, $P < 0.001$) and milk protein contents (–0.09%, $P = 0.025$), and tended to reduce fat yield (–0.03 kg/d, $P = 0.064$).

MFG Size

The range of values for fat globule size distribution was smaller in SMFG cows than in LMFG cows (Table 3 and Figure 1). These distributions show that SMFG cows had smaller sauter diameter $d_{3,2}$ (–0.47 μm, $P < 0.001$), and volume-weighted diameter $d_{4,3}$ (–0.62 μm, $P = 0.001$), and higher specific surface area (+0.30 m^2/g of fat, $P = 0.001$). Milk fat globule membrane yield was higher for SMFG cows (+260 m^2/d, $P = 0.027$, or +3.3 g/d, $P = 0.027$). The MFG membrane content in milk fat was higher for SMFG cows (+3.3‰, $P = 0.001$); however, it was lower in the milk of the same cows (–0.11‰, $P = 0.008$).

The Pt treatment reduced the globule size distribution range (–1 μm). It also led to a decrease in sauter diameter $d_{3,2}$ (–0.22 μm, $P < 0.001$), and volume-weighted diameter $d_{4,3}$ (–0.29 μm, $P < 0.001$), and an increase in the specific surface area (+0.14 m^2/g, $P = 0.005$). The MFG membrane content in milk fat was higher for cows fed the Pt diet (+1.6‰, $P = 0.005$); however, it was lower in the milk of the same cows (–0.15‰, $P < 0.001$).

Milk Nitrogen Composition and Yield

Milk from SMFG cows had lower contents of CP (–4.5 g/kg, $P = 0.002$), true protein (–4.6 g/kg, $P = 0.002$), and casein (–3.9 g/kg, $P = 0.002$; Table 4). Contents of NPN and soluble protein were similar between SMFG and

Table 3. Milk fat globule (MFG) size and MFG membrane yield and contents from cows selected for small milk fat globule size (SMFG) or large milk fat globule size (LMFG) and offered either fresh pasture (treatment Pt) or a corn silage-based diet (treatment CS)[1]

	Forage		Globule size				Effects	
	CS	Pt	SMFG	LMFG	SD	SD_{MFG}[2]	Forage	MFG
Diameter,[3] μm								
$d_{3,2}$	3.38	3.15	3.03	3.51	0.131	0.187	***	***
$d_{4,3}$	3.94	3.65	3.49	4.11	0.167	0.247	***	**
MFG membrane								
S,[4] m^2/g of fat	1.95	2.09	2.18	1.87	0.087	1.240	**	**
Yield, m^2/d	1,971	2,023	2,127	1,867	108.7	147.6	NS	*
Yield, g/d	21.7	22.2	23.4	20.5	1.20	1.62	NS	*
Content in fat, ‰	21.5	23.0	23.9	20.6	0.96	1.35	**	**
Content in milk, ‰	0.96	0.86	0.86	0.97	0.026	0.054	***	*

[1]No interaction between forage type and MFG cows was observed.

[2]SD_{MFG} = Standard deviation to test the MFG effect.

[3]$d_{3,2}$ = Sauter diameter $d_{3,2} = \Sigma(N_i \times d_i^3)/\Sigma(N_i \times d_i^2)$ where N_i is the number of fat globules in a size class i of diameter d_i; $d_{4,3}$ = Average diameter $d_{4,3} = \Sigma(N_i \times d_i^4)/\Sigma(N_i \times d_i^3)$ where N_i is the number of fat globules in a size class i of diameter d_i.

[4]S = Specific area $S = 6/(\rho \times d_{3,2})$ where ρ is the particle density (0.92 for milk fat).

*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$.

LMFG cows. The amount of NPN secreted daily in milk was higher in SMFG cows than LMFG cows (+10 g/d, $P = 0.002$). The amount of CP, true protein, casein, and soluble protein secreted daily in milk tended to be higher for SMFG cows.

Treatment Pt induced lower contents of CP (–0.9 g/kg, $P = 0.038$), true protein (–0.9 g/kg, $P = 0.046$), casein (–0.7 g/kg, $P = 0.080$), and soluble protein (–0.3 g/kg, $P = 0.042$), but did not modify NPN content. However, treatment Pt increased CP, true protein, casein, NPN, and soluble protein yields (89 g/d, $P = 0.002$; 86 g/d, $P = 0.002$; 19 g/d, $P = 0.002$; 3.2 g/d, $P = 0.002$; and 16 g/d, $P = 0.003$, respectively). Neither MFG size nor feeding treatment had an effect on the casein/protein ratio (Table 4).

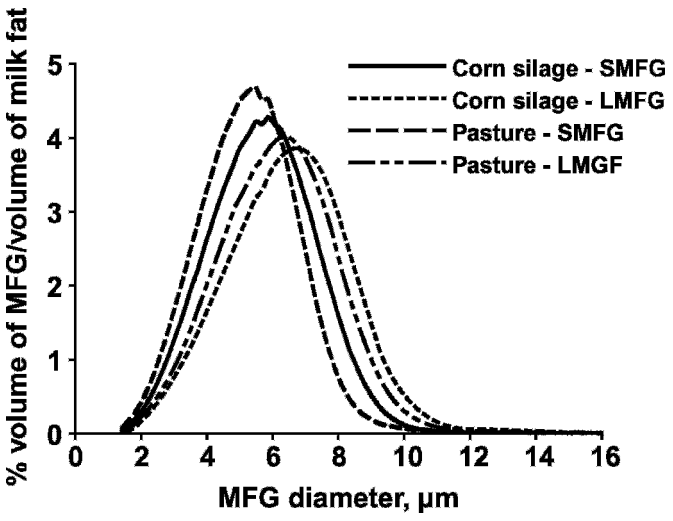

Figure 1. Milk fat globule size distribution in milk from cows selected for small milk fat globule size (SMFG) or large milk fat globule size (LMFG) and offered either fresh pasture (treatment Pt) or a corn silage-based diet (treatment CS).

Milk Calcium Content and Yield

The milk of SMFG and LMFG cows had similar contents total Ca (Table 5). However, SMFG milk had higher contents of soluble Ca (+44 mg/kg, $P = 0.004$), a greater colloidal Ca/casein ratio (+2.6 points, $P = 0.018$) and lower contents of colloidal Ca content (–72 mg/kg, $P = 0.060$). The SMFG milk had higher yields of total, soluble, and colloidal Ca than did LMFG milk (+6.9 g/d, $P = 0.005$; +2.8 g/d, $P < 0.001$; and +4.1 g/d, $P = 0.014$, respectively).

Treatment Pt resulted in lower total Ca, soluble Ca, and colloidal Ca levels (–138 mg/kg, $P < 0.001$; –26 mg/kg, $P = 0.028$; and –112 mg/kg, $P < 0.001$, respectively), but did not affect Ca yields. Treatment Pt also decreased the colloidal Ca/casein ratio (–3.4 points, $P = 0.004$).

Milk Fatty Acid Composition and Yield

Few differences were observed between SMFG and LMFG cows with respect to milk FA composition and yield (Tables 6 and 7). The SMFG cows tended to have lower percentages of saturated FA, and, more specifically, lower percentages of short even-chain FA (C4:0 to C12:0) and C18:0. The SMFG cows tended to have a higher proportion of monounsaturated FA (**MUFA**),

Table 4. Milk protein composition and yield from cows selected for small milk fat globule (MFG) size (SMFG) or large milk fat globule size (LMFG) and offered either fresh pasture (treatment Pt) or a corn silage-based diet (treatment CS)[1]

	Forage		Globule size				Effects	
	CS	Pt	SMFG	LMFG	SD	SD_{MFG}[2]	Forage	MFG
CP, g/kg	34.8	33.9	32.1	36.6	0.91	1.77	*	**
NPN, g/kg	1.5	1.4	1.5	1.4	0.11	0.05	NS	NS
True protein, g/kg	33.3	32.4	30.6	35.2	0.90	1.77	*	**
Casein, g/kg	26.7	26.0	24.4	28.3	0.82	1.56	†	**
Soluble protein, g/kg	6.6	6.4	6.2	6.8	0.20	0.92	*	NS
Casein/protein, %	80.2	80.3	79.8	80.6	0.52	2.48	NS	NS
CP, g/d	781	870	875	776	44.8	113.5	**	NS
NPN, g/d	33.7	36.9	40.3	30.3	1.62	4.05	**	**
True protein, g/d	747	833	835	745	44.7	110.1	**	NS
Casein, g/d	598	668	666	600	36.9	83.8	**	NS
Soluble protein, g/d	149	165	169	145	9.3	33.0	**	NS

[1]No interaction between forage type and MFG cows was observed.

[2]SD_{MFG} = Standard deviation to test the MFG effect

†$P \leq 0.1$; *$P \leq 0.05$; **$P \leq 0.01$.

partly due to a higher *cis*-9 C18:1 content. The SMFG cows had a higher C18:1/C18:0 ratio (+0.49, $P = 0.011$), but C16:1/C16:0 and C14:1/C14:0 ratios did not differ between SMFG and LMFG cows. The differences in the amounts of FA synthesized daily by SMFG and LMFG cows were similar to the proportional results (Table 7 and Figure 2). The SMFG cows produced (or tended to produce) fewer short even-chain FA (C4:0 to C12:0) and C18:0. The spreadability index, C16:0/C18:1 ratio, and atherogenic index (C12:0 + 4 × C14:0 + C16:0)/unsaturated FA; Ulbricht and Southgate, 1991) did not differ between SMFG and LMFG cows.

Treatment Pt increased the percentage and yield of MUFA and polyunsaturated FA (**PUFA**) at the expense of the percentage and yield of saturated FA. Treatment Pt decreased the percentage and yield of short even-chain saturated FA (C4:0 to C12:0; –2.5 points, $P < 0.001$, and –24.3 g/d, $P < 0.001$). The decrease in saturated FA was largely due to C16:0 (–12.6 points, $P < 0.001$, and –114 g/d, $P < 0.001$); C18:0 was the only even-chain saturated FA that increased in percentage and yield with treatment Pt (+3.3 points, $P < 0.001$, and +24.3 g/d, $P < 0.001$). The increase in the percentage and yield of MUFA with treatment Pt was primarily due to an increase in the percentage and yield of *cis*-9 (+*trans*-13) C18:1 (+7.0 points, $P < 0.001$, and +52 g/d, $P < 0.001$) and *trans*-11 C18:1 (+3.44 points, $P < 0.001$, and +27.1 g/d, $P < 0.001$). Treatment Pt induced higher percentages and yields of long *cis* MUFA (*cis*-6, *cis*-13, and *cis*-15 C18:1) and *trans* MUFA (*trans*-6, *trans*-7, *trans*-8, *trans*-11, and *trans*-15 C18:1), with the exception of *cis*-11 and *trans*-10 C18:1. The increase in the percentage and yield of *trans* C18:1 was essentially due to an increase in *trans*-11 C18:1. Treatment Pt reduced

Table 5. Milk calcium contents and yield from cows selected for small milk fat globule (MFG) size (SMFG) or large milk fat globule size (LMFG) and offered either fresh pasture (treatment Pt) or a corn silage-based diet (treatment CS)[1]

	Forage		Globule size				Effects	
	CS	Pt	SMFG	LMFG	SD	SD_{MFG}[2]	Forage	MFG
Calcium, mg/kg								
Total	1,282	1,145	1,199	1,228	36.9	75.1	***	NS
Soluble	293	267	302	259	24.0	20.1	*	**
Colloidal	989	877	897	969	46.8	59.3	***	†
Colloidal/casein, ‰	37.2	33.8	36.8	34.2	2.04	1.63	**	*
Calcium, g/d								
Total	28.9	29.5	32.7	25.8	1.92	3.27	NS	**
Soluble	6.7	7.0	8.2	5.5	0.68	0.90	NS	***
Colloidal	22.2	22.6	24.5	20.3	1.84	2.41	NS	*

[1]No interaction between forage type and MFG cows was observed.

[2]SD_{MFG} = Standard deviation to test the MFG effect.

†$P \leq 0.1$; *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$.

Table 6. Milk fatty acid (FA) composition from cows selected for small milk fat globule (MFG) size (SMFG) or large milk fat globule size (LMFG) and offered either fresh pasture (treatment Pt) or a corn silage-based diet (treatment CS)[1]

	Forage		Globule size				Effects	
FA,[2] weight %	CS	Pt	SMFG	LMFG	SD	SD_{MFG}[2]	Forage	MFG
C4:0	3.6	3.4	3.6	3.4	0.26	0.26	†	NS
C6:0	2.2	1.8	1.8	2.1	0.16	0.21	***	*
C8:0	1.4	1.1	1.1	1.4	0.11	0.17	***	*
C10:0	3.3	2.7	2.8	3.3	0.30	0.41	***	*
C10:1	0.36	0.24	0.26	0.34	0.030	0.048	***	*
C11:0	0.06	0.03	0.02	0.06	0.030	0.040	*	NS
C12:0	3.9	3.1	3.2	3.8	0.40	0.51	***	†
Cis-7 C12:1	0.10	0.04	0.05	0.09	0.025	0.030	***	*
C13:0	0.12	0.09	0.09	0.12	0.029	0.037	*	NS
C14:0	11.4	10.1	10.7	10.7	0.68	0.71	**	NS
Cis-9 C14:1	1.23	0.94	1.00	1.17	0.112	0.141	***	†
C15:0	1.29	1.30	1.26	1.33	0.143	0.181	NS	NS
Iso C15:0	0.23	0.26	0.24	0.25	0.022	0.026	*	NS
Anteiso C15:0	0.56	0.70	0.63	0.64	0.078	0.109	**	NS
C16:0	38.8	26.2	32.7	32.2	1.81	2.03	***	NS
Cis-9 C16:1	2.05	1.50	1.75	1.81	0.21	0.38	***	NS
Cis-7 C16:1	0.16	0.26	0.22	0.20	0.057	0.036	**	NS
C17:0	0.55	0.63	0.60	0.58	0.069	0.048	*	NS
Iso C17:0	0.32	0.41	0.37	0.36	0.028	0.026	***	NS
Anteiso C17:0	0.68	0.70	0.68	0.70	0.064	0.074	NS	NS
C18:0	6.9	10.2	8.0	9.2	0.74	1.02	***	†
Cis-15 C18:1	0.19	0.47	0.37	0.29	0.101	0.076	***	†
Trans-15 C18:1	0.48	0.86	0.69	0.65	0.117	0.113	***	NS
Cis-13 C18:1	0.07	0.18	0.13	0.12	0.034	0.040	***	NS
Cis-11 C18:1	0.19	0.14	0.17	0.15	0.050	0.042	*	NS
Trans-11 C18:1	0.82	4.26	2.66	2.42	0.843	0.845	***	NS
Trans-10 C18:1	0.26	0.14	0.28	0.12	0.202	0.176	NS	NS
Cis-6 + *trans*-12 C18:1	0.46	0.54	0.52	0.49	0.064	0.041	**	NS
Cis-9 + *trans*-13 C18:1	15.1	22.1	19.4	17.8	1.68	1.42	***	†
Trans-6 + *trans*-7 + *trans*-8 C18:1	0.31	0.52	0.42	0.41	0.088	0.081	***	NS
Cis cis C18:2 n-6	1.32	1.42	1.46	1.28	0.247	0.163	NS	NS
Trans trans C18:2 n-6	0.19	0.52	0.36	0.35	0.064	0.051	***	NS
Cis-9,*trans*-11 CLA	0.45	1.61	1.13	0.92	0.419	0.342	***	NS
C18:3 n-3	0.16	0.78	0.50	0.44	0.077	0.051	***	†
C20:1 n-9	0.18	0.22	0.23	0.17	0.033	0.029	*	**
C20:3 n-6	0.06	0.04	0.03	0.08	0.029	0.032	NS	*
C20:4 n-6	0.13	0.11	0.12	0.12	0.011	0.024	**	NS
Short FA (C4 to C12)	15.0	12.5	12.8	14.7	0.89	1.36	***	†
Long FA (>C18)	27.4	44.2	36.6	35.1	2.75	2.80	***	NS
SFA	75.6	63.1	68.1	70.5	2.64	2.24	***	†
MUFA	22.1	32.4	28.2	26.2	2.24	1.91	***	†
PUFA	2.34	4.52	3.62	3.24	0.552	0.436	***	NS
Odd-chain FA	3.8	4.1	3.9	4.0	0.35	0.37	NS	NS
Σ *Trans* C18:1	1.92	5.78	4.08	3.62	0.892	0.918	***	NS
C14:1/C14:0	0.11	0.09	0.09	0.11	0.008	0.012	**	NS
C16:1/C16:0	0.05	0.06	0.05	0.06	0.006	0.010	NS	NS
C18:1/C18:0	2.22	2.21	2.46	1.97	0.222	0.276	NS	**
Atherogenicity index	3.66	1.93	2.72	2.87	0.312	0.272	***	NS
C16:0/C18:1	2.19	0.92	1.52	1.60	0.200	0.192	***	NS

[1]No interaction between forage type and MFG cows was observed.

[2]SFA = Saturated FA; MUFA = monounsaturated FA; PUFA = polyunsaturated FA; Atherogenicity index = sum of lauric (C12:0), palmitic (C16:0), and 4 times myristic acid (C14:0) contents divided by the unsaturated FA content.

[3]SD_{MFG} = Standard deviation to test the MFG effect.

†$P \leq 0.1$; *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$.

(or tended to reduce) the percentages and yields of MUFA with fewer than 18 carbon atoms (C10:1, *cis*-7 C12:1, *cis*-9 C14:1, and *cis*-9 C16:1), with the exception of *cis*-7 C16:1. Treatment Pt increased the percentage and yield of PUFA (+2.17 points, $P < 0.001$, and +16.7 g/d, $P < 0.001$). This increase is linked to an increase in *trans trans* C18:2n-6 (+0.33 points, $P < 0.001$, and +2.6 g/d, $P < 0.001$), *cis*-9, *trans*-11 conjugated linoleic

Table 7. Milk fatty acid (FA) yield from cows selected for small milk fat globule (MFG) size (SMFG) or large milk fat globule size (LMFG) and offered either fresh pasture (treatment Pt) or a corn silage-based diet (treatment CS)[1]

	Forage		Globule size				Effects	
FA yield,[2] g/d	CS	Pt	SMFG	LMFG	SD	SD_{MFG}[3]	Forage	MFG
C4:0	31.1	28.1	29.4	29.8	3.00	3.33	*	NS
C6:0	18.6	14.9	15.1	18.5	1.77	2.37	***	*
C8:0	11.7	9.4	9.2	11.9	0.99	1.91	***	*
C10:0	28.1	23.0	22.8	28.3	2.58	4.90	**	†
C10:1	3.1	2.0	2.2	2.9	0.20	0.59	***	*
C11:0	0.5	0.3	0.2	0.6	0.25	0.43	†	NS
C12:0	32.9	25.7	26.2	32.4	3.16	5.98	***	†
Cis-7 C12:1	0.9	0.4	0.4	0.8	0.20	0.32	***	*
C13:0	1.1	0.8	0.8	1.1	0.22	0.43	*	NS
C14:0	96.9	83.7	88.5	92.0	6.83	10.77	**	NS
Cis-9 C14:1	10.6	7.9	8.3	10.2	0.72	2.00	***	NS
C15:0	11.1	10.9	10.4	11.7	1.12	2.88	NS	NS
Iso C15:0	2.0	2.1	2.0	2.1	0.22	0.22	NS	NS
Anteiso C15:0	4.8	5.8	5.2	5.4	0.73	0.95	**	NS
C16:0	333	219	271	280	21.1	39.7	***	NS
Cis-9 C16:1	17.8	12.6	14.4	16.0	2.29	5.15	***	NS
Cis-7 C16:1	1.4	2.1	1.9	1.7	0.40	0.26	**	NS
C17:0	4.7	5.1	4.9	5.0	0.45	0.82	†	NS
Iso C17:0	2.7	3.3	3.0	3.0	0.23	0.28	***	NS
Anteiso C17:0	5.8	5.8	5.6	6.0	0.66	0.92	NS	NS
C18:0	59.3	83.6	65.7	77.2	7.51	8.51	***	*
Cis-15 C18:1	1.6	3.8	3.0	2.4	0.67	0.63	***	NS
Trans-15 C18:1	4.0	7.0	5.6	5.5	0.73	0.78	***	NS
Cis-13 C18:1	0.6	1.4	1.1	1.0	0.28	0.29	***	NS
Cis-11 C18:1	1.6	1.1	1.4	1.3	0.39	0.31	**	NS
Trans-11 C18:1	6.9	34.1	21.1	19.8	6.32	5.34	***	NS
Trans-10 C18:1	2.2	1.1	2.2	1.1	1.40	1.25	†	NS
Cis-6 + *trans*-12 C18:1	3.9	4.5	4.2	4.2	0.31	0.58	**	NS
Cis-9 + *trans*-13 C18:1	129	181	159	152	9.9	17.8	***	NS
Trans-6 + *trans*-7 + *trans*-8 C18:1	2.6	4.2	3.4	3.5	0.80	0.57	**	NS
Cis cis C18:2 n-6	11.2	11.6	11.9	11.0	1.65	1.28	NS	NS
Trans trans C18:2 n-6	1.6	4.2	2.9	2.9	0.43	0.36	***	NS
Cis-9,*trans*-11 CLA	3.8	12.9	9.0	7.7	2.97	2.26	***	NS
C18:3 n-3	1.3	6.4	4.0	3.7	0.42	0.47	***	NS
C20:1 n-9	1.6	1.8	1.9	1.5	0.24	0.39	*	NS
C20:3 n-6	0.6	0.3	0.3	0.6	0.28	0.27	†	*
C20:4 n-6	1.1	0.9	1.0	1.0	0.07	0.11	***	NS
Short FA (C4 to C12)	127.8	104.6	106.2	126.2	8.93	18.25	***	†
Long FA (>C18)	234	361	298	297	16.1	28.5	***	NS
SFA	647	524	563	608	35.6	69.9	***	NS
MUFA	189	265	230	224	10.8	27.4	***	NS
PUFA	20.0	36.6	29.2	27.3	3.22	2.77	***	NS
Odd-chain FA	32.7	34.2	32.1	34.8	3.03	6.25	NS	NS
Σ *trans* C18:1	16.2	46.4	32.6	30.0	6.13	5.34	***	NS

[1]No interaction between forage type and MFG cows was observed.

[2]SFA = Saturated FA; MUFA = monounsaturated FA; PUFA = polyunsaturated FA.

[3]SD_{MFG} = Standard deviation to test the MFG effect.

†$P \leq 0.1$; *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$.

acid (CLA; +1.16 points, $P < 0.001$, and +9.1 g/d, $P < 0.001$), and C18:3n-3 (+0.62 points, $P < 0.001$, and +5.1 g/d, $P < 0.001$). Very long chain PUFA (C20 and C22) were only slightly affected by the treatments. Odd-chain FA (C15:0 and anteiso C17:0) were not modified by the treatments. However, treatment Pt increased the percentage and yield of their isomers, *iso* C15:0, *anteiso* C15:0, C17:0, and *iso* C17:0. Treatment Pt reduced (or tended to reduce) the percentage and yield of short odd-chain FA (C11:0 and C13:0). The C18:1/C18:0 and C16:1/C16:0 ratios were not affected by the treatments. The C14:1/C14:0 ratio decreased when cows received the treatment Pt. The atherogenicity index was improved with treatment Pt (–1.72, $P < 0.001$). Treatment Pt also resulted in lowering the spreadability index (–1.27, $P < 0.001$).

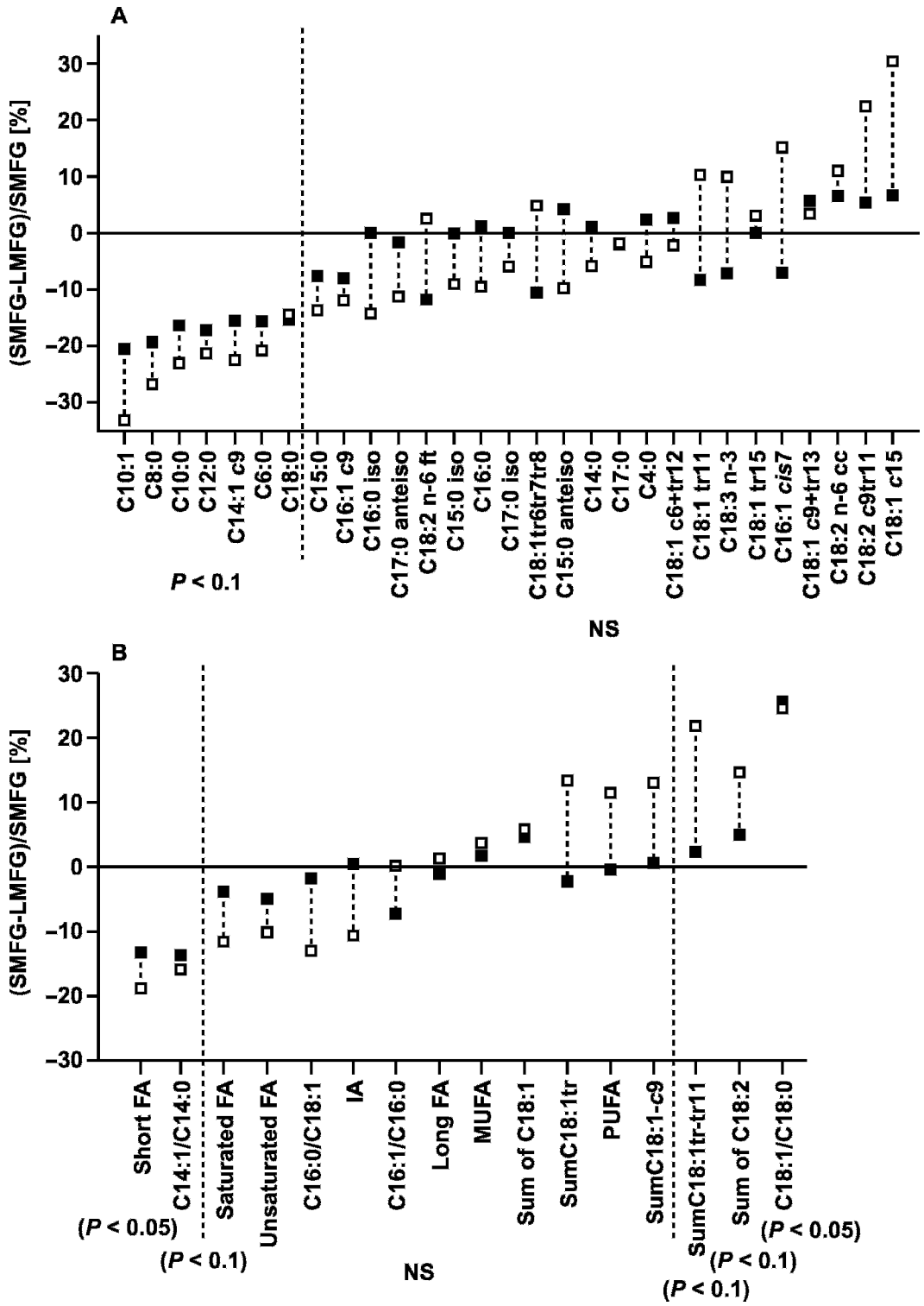

Figure 2. Relative compositional difference based on milk fatty acid (FA) composition between cows selected for small milk fat globule size (SMFG) or large milk fat globule size (LMFG) and offered either fresh pasture (treatment Pt, □) or a corn silage-based diet (treatment CS, ■). MUFA = monounsaturated FA; PUFA = polyunsaturated FA; c = *cis;* tr = *trans*.

DISCUSSION

Differences Between SMFG and LMFG Cows

Milk, Protein and Fat Yields, Protein and Fat Contents. Higher yields of milk and protein were observed in SMFG cows. These results indicate that milk synthesis seems to be more active in the epithelial cells of the SMFG cows. The SMFG cows produced more membrane material than did the LMFG cows. This result shows, for the first time, a positive relationship between the quantity of membrane and the quantity of secreted milk. Thus, the ability to produce membrane material may be the reason for high cell traffic and therefore the ability to synthesize and secrete milk and protein.

The quantity of milk fat secreted into the milk was identical for SMFG and LMFG cows; however, SMFG cows secreted it as smaller MFG, which require a larger quantity of membrane. In our study, there was no relationship between MFG size and quantity of milk fat secreted; this is not consistent with the positive correlation reported by Wiking et al. (2004). As the proportion of milk fat is standardized in most dairy products, there are more MFG and a higher membrane content in the products made from milk of SMFG cows. It may also be possible to modify the technological properties of SMFG milk fat: 1) better incorporation of MFG in cheese, 2) higher resistance to churning, and 3) more extensive water retention by the membrane, resulting in less firmness but also better storage ability (Michalski et al., 2002).

Milk from SMFG cows had lower protein and fat contents. This is probably due to a dilution of the protein and fat secreted into a higher quantity of milk. Our results show a positive correlation between MFG size and fat content, as reported by Wiking et al. (2004). In our study, this correlation was probably a result of the membrane and milk synthesis capabilities of the cows: given the same milk fat yield, the greater the synthesis activities of membrane and milk, the smaller the MFG.

FA Composition. Figures 2a and 2b present the differences in the FA composition of SMFG and LMFG cows assigned to either the Pt or CS treatments. The FA composition of milk from SMFG cows tended to contain less short-chain FA and C18:0 and more unsaturated FA than that of LMFG cows. Unlike the studies of Timmen and Patton (1988), Briard et al. (2003), and Fauquant et al. (2005), who separated MFG populations according to size by skimming or microfiltration, our research, like that of Wiking et al. (2004), compared the FA composition of MFG populations of naturally different sizes, obtained from genetically different cows. The variation in FA composition observed in our study could be due to differences in milk fat synthesis among SMFG and LMFG cows. Milk fat synthesis activity could differ in 2 ways: 1) enhanced elongation activity, which would explain the low levels of short-chain FA; and 2) higher desaturation activity resulting in a higher ratio of C18:1/C18:0. Fauquant et al. (2005) reported that the MFG membrane and the MFG core have the same FA composition. Thus, in our study, it is not possible to link the differences in milk fat composition among SMFG and LMFG cows to the difference observed in MFG membrane content in milk fat.

Effects of Diet

Milk Yield and Proportion and Secretion of Milk Fat and Nitrogen. The Pt treatment increased milk and protein yields and decreased the protein and fat contents compared with the CS treatment. Previous work has shown that milk yield may decrease or remain stable, that milk fat content may decrease and that milk protein content may either increase or decrease for cows given pasture rather than a corn silage diet (Bargo et al., 2003; Schroeder et al., 2003). In our study, increases in milk and protein yields can be explained by higher energy intake with the Pt treatment, which provided 2.7 Mcal of NE_L/d more than the CS treatment. According to the relationship between energy intake and milk yield established by Coulon and Rémond (1991), such a difference in the amount of energy intake is likely to induce an increase in milk yield ranging from 1 to 3.3 kg/d. However, the increase in protein yield was not sufficient enough to offset the increase in milk yield. Therefore, a decrease was observed in the contents of the various nitrogen and protein fractions due to a dilution effect.

Milk fat synthesis tended to decrease with the Pt treatment. This is consistent with the previously cited research and may be explained by an inhibiting effect on the de novo synthesis of long-chain unsaturated FA (Chilliard et al., 2000). In addition to the decrease in milk fat secreted, there was an increase in milk yield, leading to a considerable reduction in fat content with the Pt treatment due to a dilution effect.

Properties of MFG. The Pt treatment led to long-chain FA and unsaturated FA percentages at the expense of short- and medium-chain FA and saturated FA. The effect of diet on the FA profile of milk is consistent with the literature (Chilliard et al., 2001; Hurtaud et al., 2002a; Schroeder et al., 2003). The fat in pasture (2 to 3% of DM) is primarily composed of C18:3, whereas the fat in corn is essentially made up of C18:2 (Schroeder et al., 2004). It is therefore likely that given similar dehydrogenation activity in the rumen, a larger proportion of C18:3 and its dehydrogenation products, *trans*-11 C18:1 and C18:0 (Sauvant and Bas, 2001),

pass through the rumen and reach the udder. The Pt treatment can explain the higher percentages of C18:3n-3, *trans*-11 C18:1, C18:0, and, hence the presence of their desaturation products, *cis*-9 C18:1 and *cis*-9, *trans*-11 CLA. Specifically, about 30% of *trans*-11 C18:1 is desaturated to *cis*-9, *trans*-11 CLA. Ninety percent of *cis*-9, *trans*-11 CLA has this metabolic origin in the Pt treatment (Kay et al., 2004). The remaining 10% is accounted for by *cis*-9, *trans*-11 CLA, which is produced by the incomplete biohydrogenation of C18:3 in the rumen. There is evidence that some long-chain unsaturated FA reduce both de novo synthesis and the uptake of preformed FA (Chilliard et al., 2000). Thus, an increase in their concentrations in the mammary epithelial cells is responsible for reducing synthesis activity. This situation results in a decrease in medium-chain FA (C8:0 to C16:0; Chilliard et al., 2000).

The Pt treatment also induced an increase in the percentage of FA of nutritional interest (C18:3n-3, *cis*-9, *trans*-11 CLA, *cis*-9 C18:1, *trans*-11 C18:1), which in turn considerably lowered the atherogenic index (–50%). The effect of treatment Pt on the nutritional quality of fat is consistent with the literature (Chilliard et al., 2001). Nevertheless, the Pt treatment increased the *trans* FA content, even if *trans*-11 C18:1 is not taken into account. The increase in the *trans* FA content is associated with a significant decrease in saturated FA, the latter conferring a nutritional benefit.

Milk fat globule size varied little with the treatment Pt, which is consistent with the conclusions of Mulder and Walstra (1974) and Hurtaud et al. (2002a). The decrease in diameter at the maximum peak of the population distribution (mode) in $d_{3,2}$ and $d_{4,3}$ is due to a specific effect of pasture, regardless of its form: fresh grass (–0.21 μm; Hurtaud et al., 2002a), grass silage (–0.27 μm; Couvreur et al., 2004b), big bale haylage (–0.28 μm; Couvreur et al., 2004a), or hay (–0.38 μm; Hurtaud et al., 2002b).

As reported by Wiking et al. (2004), the globule size profiles of SMFG and LMFG cows differ in shape. All the cows fed pasture or corn silage secreted MFG with a diameter of less than 1 μm. However, the diameter at the upper end of the MFG distribution is smaller with the Pt treatment (12.5 vs. 13.8 μm for treatment CS). There is, therefore, a smaller range of globule sizes in cows fed pasture (distribution decreased in interval and mode).

CONCLUSIONS

Our study indicates that diet and selection can be used to change MFG composition and size. It also demonstrates that the effects of diet and selection are additive. Pasture may improve the nutritional qualities of fat while increasing membrane concentration and modifying the FA profile. Assuming that selecting cows for MFG size is possible, it may be possible to improve the nutritional qualities and change the technological properties of milk fat in a given dairy product.

Our results indicate that cows secreting SMFG may have a potential higher mammary metabolic activity than those secreting LMFG. Nevertheless, research on the specific cellular mechanisms involved appears necessary for a better understanding of these differences.

ACKNOWLEDGMENTS

The authors gratefully acknowledge La Région Bretagne, Gala Association and Arilait Recherches for their financial support and the lipids committee of the European Centre for Dairy Research and Training (CEREL, Rennes). They specially thank INRA UMR STLO (Rennes) for MFG size measurements. Warm thanks are extended to the farm staff of Méjusseaume (INRA UMR PL, Le Rheu) for cattle measurements, to C. Beuve, a DUT student, and to A. Brasseur and I. Jicquel (INRA UMR PL, Saint Gilles) for their technical assistance. The authors also extend appreciation to D. Catheline (Laboratoire de Biochimie, INRA.-Agrocampus, Rennes) for FA analysis and to Melanie Guedenet and Josephine Pithon for the English translation and reading.

REFERENCES

Association Française de Normalisation. 1985. Aliments des animaux. Dosage de l'azote en vue de calcul de la teneur en protéines brutes. Pages 87–93 in Association Française de Normalisation, Paris, France.

Avramis, C. A., H. Wang, B. W. McBride, T. C. Wright, and A. R. Hill. 2003. Physical and processing properties of milk, butter, and Cheddar cheese from cows fed supplemental fish meal. J. Dairy Sci. 86:2568–2576.

Bargo, F., L. D. Muller, E. S. Kolver, and J. E. Delahoy. 2003. Invited review: Production and digestion of supplemented dairy cows on pasture. J. Dairy Sci. 86:1–42.

Briard, V., N. Leconte, F. Michel, and M. C. Michalski. 2003. The fatty acid composition of small and large naturally occurring milk fat globules. Eur. J. Lipid Sci. Technol. 105:677–682.

Chilliard, Y., A. Ferlay, R. M. Mansbridge, and M. Doreau. 2000. Ruminant milk fat plasticity: Nutritional control of saturated, polyunsaturated, *trans* and conjugated fatty acids. Ann. Zootech. 49:181–205.

Chilliard, Y., A. Ferlay, and M. Doreau. 2001. Effect of different types of forages, animal fat or marine oils in cow's diet on milk fat secretion and composition, especially conjugated linoleic acid (CLA) and polyunsaturated fatty acids. Livest. Prod. Sci. 70:31–48.

Coulon, J. B., and B. Rémond. 1991. Variations in milk output and milk protein content in response to the level of energy supply to the dairy cow: A review. Livest. Prod. Sci. 29:31–47.

Couvreur, S., C. Hurtaud, L. Delaby, and J. L. Peyraud. 2004a. Effect of haylage or maize silage based diets with or without energy restriction on milk fat properties. Pages 1142–1144 in Proc. Grassl. Sci. Eur. Vol. 9. Eur. Grassl. Fed., Luzern, Switzerland.

Couvreur, S., C. Hurtaud, L. Delaby, and J. L. Peyraud. 2004b. Influence de différentes races et régimes sur les caractéristiques de la matière grasse du lait. Renc. Rech. Ruminants 11:107.

Delagarde, R., and M. O'Donovan. 2005. Modelling of herbage intake and milk production by grazing dairy cows. Pages 89–104 in Proc. XXth Int. Grassl. Congr., Utilisation of grazed grass in temperate animal systems. J. J. Murphy, ed. Wageningen Academic, Wageningen, the Netherlands.

Fauquant, C., V. Briard, N. Leconte, and M. C. Michalski. 2005. Differently sized native milk fat globules separated by microfiltration: Fatty acid composition of the milk fat globule membrane and triglyceride core. Eur. J. Lipid Sci. Technol. 107:80–86.

Heid, H. W., and T. W. Keenan. 2005. Intracellular origin and secretion of milk fat globules. Eur. J. Cell Biol. 84:245–258.

Hurtaud, C., L. Delaby, and J. L. Peyraud. 2002a. Evolution of milk composition and butter properties during the transition between winter-feeding and pasture. Pages 574–575 in Proc. Grassl. Sci. Europe. Vol. 7. Eur. Grassl. Fed., La Rochelle, France.

Hurtaud, C., H. Goudédranche, L. Delaby, B. Camier-Caudron, and J. L. Peyraud. 2002b. Effet de la nature du régime hivernal sur la qualité du beurre et de l'emmental. Renc. Rech. Ruminants 9:369.

Institut National de la Recherche Agronomique (INRA). 1989. Ruminant Nutrition: Recommanded Allowances and feed Tables. R. Jarrige, ed. John Libbey, Eurotext, London, UK.

Kay, J. K., T. R. Mackle, M. J. Auldist, N. A. Thomson, and D. E. Bauman. 2004. Endogenous synthesis of *cis*-9, *trans*-11 conjugated linoleic acid in dairy cows fed fresh pasture. J. Dairy Sci. 87:369–378.

Keenan, T. W., and D. P. Dylewski. 1995. Intracellular origin of milk lipid globules and the nature and structure of the milk lipid globule membrane. Pages 89–130 in Advanced Dairy Chemistry Vol. 2: Lipids. 2 ed. P. F. Fox, ed. Chapman and Hall, London, UK.

Michalski, M. C., V. Briard, and F. Michel. 2001. Optical parameters of milk fat globule for laser light scattering measurements. Lait 81:787–796.

Michalski, M. C., R. Cariou, F. Michel, and C. Garnier. 2002. Native vs. damaged milk fat globules: Membrane properties affect the viscoelasticity of milk gels. J. Dairy Sci. 85:2451–2461.

Michalski, M. C., J. Y. Gassi, M. H. Famelart, N. Leconte, B. Camier, F. Michel, and V. Briard. 2003. The size of native milk fat globules affects physico-chemical and sensory properties of Camembert cheese. Lait 83:131–143.

Michalski, M. C., B. Camier, V. Briard, N. Leconte, J. Y. Gassi, H. Goudedranche, F. Michel, and J. Fauquant. 2004. The size of native milk fat globules affects physico-chemical and functional properties of Emmental cheese. Lait 84:343–358.

Mulder, H., and P. Walstra. P. 1974. The fat dispersion. Pages 54–66 in The Milk Fat Globule. Emulsion science as applied to milk products and comparable foods. H. Mulder and P. Walstra, ed. Center for Agricultural Publishing and Documentation, Wageningen, the Netherlands.

O'Mahony, J. A., M. A. E. Auty, and P. L. H. McSweeney. 2005. The manufacture of miniature Cheddar-type cheeses from milks with different fat globule size distributions. J. Dairy Res. 72:338–348.

Rowland, S. J. 1938. The determination of the nitrogen distribution in milk. J. Dairy Res. 9:42–46.

SAS Institute. 1990. SAS User's Guide: Statistics. Version 6. SAS Inst, Inc., Cary, NC.

Sauvant, D., and P. Bas. 2001. La digestion des lipides chez le ruminant. INRA Prod. Anim. 14:303–310.

Schroeder, G. F., J. E. Delahoy, I. Vidaurreta, F. Bargo, G. A. Gagliostro, and L. D. Muller. 2003. Milk fatty acid composition of cows fed a total mixed ration or pasture plus concentrate replacing maize with fat. J. Dairy Sci. 86:3237–3248.

Schroeder, G. F., G. A. Gagliostro, F. Bargo, J. E. Delahoy, and L. D. Muller. 2004. Effects of fat supplementattion on milk production and composition by dairy cows on pasture: A review. J. Dairy Sci. 86:1–18.

Timmen, H., and S. Patton. 1988. Milk fat globules: Fatty acid composition, size and in vivo regulation of fat liquidity. Lipids 23:685–689.

Van Soest, J. P., J. B. Robertson, and B. A. Lewis. 1991. Methods of dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrtion. J. Dairy Sci. 71:1587–1597.

Wiking, L., L. Björck, and J. H. Nielsen. 2003. Influence of feed composition on stability of fat globules during pumping of raw milk. Int. Dairy J. 13:797–803.

Wiking, L., J. Stagsted, L. Björck, and J. H. Nielsen. 2004. Milk fat globule size is affected by fat production in dairy cows. Int. Dairy J. 14:909–913.

Xiong, Y. L., and J. E. Kinsella. 1991. Influence of fat globule membrane composition and fat type on the rheological properties of milk based composite gels. II. Results. Milchwissenschaft 46:207–212.