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# The Linear Relationship Between the Proportion of Fresh Grass in the Cow Diet, Milk Fatty Acid Composition, and Butter Properties

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## ABSTRACT

Fresh grass in the cow diet improves the rheological and nutritional properties of butter. However, the relationship between the proportion of fresh grass in the diet and these properties is still unknown. The objective of the study was to determine the relationship between the proportion of fresh grass in the diet and the properties of milk and butter. Four groups of 2 cows were fed 4 isoenergetic diets characterized by increasing amounts of fresh grass (0, 30, 60, and 100% dry matter of forage) according to a Youden square design. Energy levels were similar among all diets. Thus, no effect of mobilization was observed and the results were only due to the proportion of fresh grass in the diet. Milk yield linearly increased with the proportion of fresh grass in the diet (+0.21 kg/d per 10% of grass). Fat yield remained unchanged. Thus, by effect of dilution, increasing the proportion of fresh grass in the diet induced a linear decrease in fat content. Milk fat globule size decreased by 0.29 μm when the proportion of grass reached 30% in the diet. Increasing the proportion of fresh grass in the diet induced a linear increase in unsaturated fatty acids percentages at the expense of saturated fatty acids. Relationships were +0.38, +0.12, +0.05 and −0.69 points/10% of fresh grass in the diet for C18:1 *trans*-11, C18:2 *cis*-9,*trans*-11, C18:3n-3, and C16:0, respectively. These modifications in fatty acid composition, and in particular in the spreadability index, C16:0/C18:1, were responsible for linear decreases in final melting temperature and solid fat content in butter fat, perceived in sensory analysis by a linear decrease in firmness in mouth. The nutritional value of butter was also linearly improved by the proportion of fresh grass in the diet by halving the atherogenicity index.

**Key words:** fresh grass, milk fatty acid, butter properties, milk fat globule

## INTRODUCTION

Butter is often criticized for its poor nutritional value and spreadability compared with margarines (Brodin, 1989). Studies have shown that spreadability is positively correlated to the percentage of unsaturated fatty acids (**UFA**) in butter fat (Wood et al., 1975; Banks and Christie, 1990; Hillbrick and Augustin, 2003). On the other hand, a recent study by Goudédranche et al. (2000) showed that reducing the milk fat globule (**MFG**) size from 3 to 2 μm improved butter spreadability. This may be because of an increase in water retention by the MFG membrane, which, in turn, induces a reduction in butter DM (Michalski et al., 2002).

At grazing, milk from dairy cows has higher UFA levels (which results in a more spreadable butter) compared with milk from cows fed indoors with a corn silage diet (Cullinane et al., 1984; Hurtaud et al., 2002a; Schroeder et al., 2003; Ledoux et al., 2005). However, this high level of UFA in milk could cause flavor defects due to oxidation (Hurtaud et al., 2002a). Finally, although it was recently proven that grass induced a reduction of about 0.3 to 0.5 μm in MFG size, it has not been determined if the reduction was reflected in the functional properties of butter (Hurtaud et al., 2002b).

Kelly et al. (1998), Agenäs et al. (2002), and Hurtaud et al. (2002a) suggested that the effects of grass on milk fat properties and sensory and nutritional characteristics of butter are established as soon as the transition period begins, before the animals are at full grazing. Thus, a diet totally composed of grazed grass does not seem necessary to induce significant modifications of milk composition and butter quality. If it were confirmed, this would indicate that a few hours of grazing per day could be sufficient to modulate the effects of a corn silage diet and thus, to reduce fully or partly, the seasonal effects on sensory and functional qualities of winter and summer butters.

The objective of this study was to investigate the effect of replacing corn silage with increasing proportions of fresh cut grass in the cow diet on milk characteristics and on the functional and sensory properties of butter.

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**Table 1.** Chemical composition of forages and supplements

| | Corn silage | Grass[1] | Soybean | Cereal concentrate[2] |
|---|---|---|---|---|
| DM, wt % | 42.8 | 17.7 ± 3.24 | 89.7 | 90.5 |
| NDF, g/kg of DM | 370 | 492 ± 24.6 | 141 | 232 |
| CP, g/kg of DM | 72 | 199 ± 9.6 | 480 | 118 |
| PDIE,[3] g/kg of DM | 72 | 105 ± 3.3 | 239 | 98 |
| PDIN,[4] g/kg of DM | 44 | 125 ± 5.9 | 341 | 79 |
| $NE_L$, kg of DM | 1.57 | 1.77 ± 0.03 | 2.02 | 1.84 |
| Ether extract, g/kg of DM | 32 | 38 ± 1.7 | 30 | 43 |

[1]Average value of 4 grass samples (mean ± SD; 1 per period).

[2]Mixture of wheat (20% DM), barley (20% DM), corn (20% DM), beet pulp (20% DM), finest bran wheat (15% DM), beet molasses (3% DM), vegetable oil (1% DM), and salt (1% DM), completed by 250 g of 5-18-10 minerals (P-Ca-Mg).

[3]PDIE = Digestible protein in the small intestine supplied by microbial protein from rumen-fermented OM (INRA, 1989)

[4]PDIN = Digestible protein in the small intestine supplied by microbial protein from rumen-degraded OM (INRA, 1989)

## MATERIALS AND METHODS

### *Experimental Design*

The trial was conducted on 8 multiparous fistulated Holstein cows (4 groups of 2 cows). On average, animals were at 150 d in lactation (±19 d), produced 33.2 kg/d of milk (±1.3 kg/d) characterized by 4.02% fat content (±0.49%) and 3.00% (±0.18%) protein content. During 4 experimental 2-wk periods (14 d) from April 28, 2003 to June 22, 2003, 4 treatments represented by 0 (**G0**), 30 (**G30**), 60 (**G60**), and 100% (**G100**) of fresh cut grass to replace corn silage were applied according to a Youden square. A Latin square design could not be applied because going from 0 to 100% of fresh cut grass in the diet (and vice versa) in a transition of only 1 wk was not feasible. Thus, G0 and G100 were applied to the same groups during 2 periods. One week of diet transition (7 d) and 1 wk of measurements (7 d) constituted each experimental period.

### *Treatments and Feeding*

Based on chemical composition [NDF, CP, energy, digestible proteins in the intestine (**PDI**)] of forages and concentrates, offered diets were calculated to be as isoenergetic and isoproteic as possible (Table 1). Before commencement of the trial, cows were fed ad libitum a diet of 50:50 corn silage:fresh grass as forage and a 50:50 soybean:cereal concentrate as supplementation. Dry matter intakes were measured individually to establish the amounts of forage (grass or corn silage) and concentrates offered during the experiment. In all treatments, the fresh grass was from the same meadow, sown as a combination of perennial ryegrass and white clover in autumn 1998, but poor in white clover in 2003 (10 to 15% DM). The meadow was divided in 4 paddocks; 1 paddock was used per experimental period. Each paddock was mowed 21 d before the beginning of the experimental period to maintain the regrowth stage of fresh grass between 28 and 35 d during the weeks of measurements. After mowing, paddocks were fertilized with ammonium nitrate at a rate of 60 kg of N/ha. Treatments G0, G30, G60, and G100 were supplemented by 3 kg of a mixture composed of 3:0, 2:1, 1:2, and 0:3 soybean:cereal concentrate, respectively. Cereal concentrate was a mixture of wheat (20% DM), barley (20% DM), corn (20% DM), beet pulp (20% DM), finest bran wheat (15% DM), beet molasses (3% DM), vegetable oil (1% DM), and salt (1% DM). The diet was completed by 250 g of 5-18-10 minerals (P-Ca-Mg).

At each experimental week, 2 daily samples (50 g) of corn silage and the soybean and cereal concentrate were taken. They were pooled by period, and preserved at –20°C. Dry matter, total N, ether extract, and mineral contents were analyzed. Two daily samples (700 g) of fresh grass were taken in each experimental week, and dried at 80°C for 48 h to determine the DM content and to measure the total N, ether extract, and minerals. (Apper-Bossard et al., 2006).

Animals were weighed at the beginning and at the end of the trial. Forage and concentrate intakes were measured at each meal to evaluate feed intake, energy and protein balance.

### *Sampling Schedule and Procedure*

***Milk.*** Cows were milked at 0700 and 1800 h during the trial. Milk yield was recorded at each milking using electronic flow meters (Metatron 21, Westfalia, Germany). Fat and protein contents were determined at each milking between d 8 and 13 by infrared analysis (Milkoscan, Foss Electric, Hillerød, Denmark).

***Physicochemical Properties of Milk Fat Globules.*** A 1-L sample was taken individually at the evening and morning milkings on d 10. The 2 samples were pooled per cow according to milk production of the sampling day.

Fat globule size distribution was determined in milk and in cream used for the manufacture of butter by laser light scattering using a Mastersizer 2000 (Malvern Instruments, Malvern, UK) with 2 different wavelengths (He/Ne laser: 633 nm; electroluminescent diode: 466 nm). The obscuration rate for measurements was similar between samples, and fixed at 10%. Samples were measured by diluting with 35 m*M* EDTA/NaOH, pH 7.0, buffer (1:1 vol.), to dissociate casein micelles, then dispersing in a sample unit containing 100 mL of 0.1% (w/vol) SDS solution in purified water. The refractive indexes of MFG in water were 1.458 at 633 nm and 1.460 at 466 nm (Michalski et al., 2001). From the size distribution (in % vol), the diameter at the maximum of the distribution peak (mode), the average diameters $d_{3,2} = \sum(N_i \times d_i^3)/\sum(N_i \times d_i^2)$, and $d_{4,3} = \sum(N_i \times d_i^4)/\sum(N_i \times d_i^3)$, and the specific area $S = 6/(\rho \times d_{3,2})$ were calculated by the Malvern software [where $N_i$ is the number of fat globules in a size class I of diameter $d_i$, and $\rho$ is the particle density (0.92 for milk fat)]. An estimation of the surface area of membrane synthesized daily was made by multiplying the specific surface area by the fat production.

Fatty acid (**FA**) composition was determined in milk stored at –20°C. The measurements were performed on 0.5 mL of milk. Lipids were extracted from milk with 0.5 mL of ethanol 96.2°/Hydro-chloric 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). 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 were methylated with 1 mL of boron trifluoride (20% vol in methanol) 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 inner diameter, BPX 7; SGE, Villeneuve-St-Georges, France) with a stationary phase of 70% cyanopropylpolysilphenylene-siloxane (0.25-μm film thickness). Helium was used as gas vector ($10^5$ Pa). The column temperature program started at 60°C for 7 min, increasing at 7°C/min to 150°C, then increasing at 0.7°C/min to 210°C, and holding at 210°C for 10 min. The flame-ionization detector temperature was 260°C. Identification of FA methyl ester peaks was based upon 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, 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) of our samples.

***Butter Manufacture.*** Butter was manufactured for each group during the 4 experimental periods using an experimental churn at the Centre d'Expérimentation et de Technologie Agro-Alimentaire (CETAA, Rennes, France). One cow from group 4 died during trial. Thus, milk from one cow was not sufficient for butter manufacture in periods 3 and 4 for group 4. Fourteen butters were manufactured during the study. For a manufacture, the amount of milk corresponding to 3 d of milking was stored at 4°C in individual tanks and then brought to CETAA. Milk was skimmed at 40°C using a cream separator (Elecrem 3, Elecrem, Chatillon, France). Cream was standardized at 350 g/kg of fat and pasteurized (80°C for 20 s). After pasteurization, cream was cooled as rapidly as possible at 4°C. The physical ripening was as follows: i) storage for about 5 h at 4°C, and ii) storage for 18 h at 15°C. Cream was inoculated with a starter at the beginning of the physical ripening to develop flavor, crystallize the fat, and reduce pH to a value of 5.2. Eight to 10 kg of cream were sampled, and then churned at 10°C in an experimental churn (Elba 30, Elecrem) until butter kernels formed. The buttermilk was separated from the butter kernels. Butter was washed twice with cold water (10°C) in the churn. Washed butter was worked in the churn into a homogeneous mass. Butter was packaged by hand with a spatula into 500-mL cream plastic cups (diameter: 10 cm, depth: 8 cm). Four cups were conserved at –20°C for fat extraction, FA composition, and thermal properties of butter fat; 10 cups were conserved at 4°C for rheological properties and sensory analysis.

***Physicochemical Properties of Butter.*** Dry matter and fat content were measured on the samples conserved at –20°C according to the reference method (ISO, 1995; method no. EN ISO 3727). Dry matter was measured by recording the mass lost by a sample of butter of 5 ± 1 g during drying in an oven at 102 ± 2°C for 15 h. Fat was extracted from dry butter previously obtained using 60 mL of n-hexane. The extraction residue (nonfat DM) was dried at 102 ± 2°C until it reached a constant mass. Butter DM was calculated by dividing the difference between the original sample mass and water mass by the original sample mass. Butter fat content was calculated as follows:

$$fatcontent = \frac{[m_{butter} - (m_{water} + m_{nonfat})]}{m_{butter}} \times 100.$$

The cups of butter stored at –20°C were analyzed for FA composition by the same method as that used for milk samples.

Butter samples (one sample corresponds to a cup of butter) were tested for resistance to penetration using a universal testing machine (Instron, model 4501, Norwood, MA) with IX series software (Instron, Norwood, MA), equipped with a 1-kN load cell and a 90° cone in a room maintained at 18°C. Measurements were taken on 3 samples of butter conserved at 4°C during 15 d. The butters were taken out of the cooler (at 4°C) just before measurement. One measurement per sample was performed. The sample was taken out of the cup for measurement to avoid measuring resistance to penetration related to the resistance of the cup. The 90° cone sank into the samples at 15 mm/min until 18 mm of penetration (26% penetration of the total depth of the sample). The rheological parameters were the forces (N) measured at 5, 10, and 18 mm of penetration.

The melting and cooling properties of butter fat were examined by differential scanning calorimetry (**DSC**) using a TA Q1000 calorimeter (TA Instruments, Saint Quentin en Yvelines, France). Calibration was carried out using indium (melting temperature = 156.5985°C, $\Delta$H melting = 28.57 ± 0.17 J/g; TA Instruments). Anhydrous butter fat was extracted from 0.5 g of butter stored at –20°C using the following procedure. Approximately 20 mL of hexane:isopropanol (3:2 vol:vol) mix was added to the butter. The mixture was vortexed and centrifuged for 10 min at 1,200 rpm. The upper organic phase was separated and added to a second organic phase obtained by a second extraction of the lower phase using 7 mL of hexane, and the solvent was evaporated from the pooled fraction under vacuum until it reached a constant weight. About 4 to 6 mg of butter fat was weighed into a hermetic aluminum 50-μL pan (Waters S.A., Saint Quentin en Yvelines, France). An empty hermetic aluminum pan was used as reference. Measurements were performed in triplicate for each butter fat. The samples were heated at 60°C for 5 min, cooled at 2°C/min from 60 to –40°C, and then heated at 2°C/min from –40 to 60°C. Low melting fraction (**LMF**), medium melting fraction (**MMF**), and final melting (**T offset**) temperatures were measured from melting profiles. The temperature of the beginning of crystallization (**T onset**) and of each peak was measured from cooling profiles. The solid fat content profile of butter fat was determined by calculating the cumulative area under the melting curve between –40 and +60°C.

For sensory analysis, 5 cups of butter per manufactured butter (n = 5 × 14) stored at 4°C were sent to Ecole Nationale d'Industrie Laitière et des Industries Agroalimentaires (Surgères, France) by refrigerated transport (4°C) 2 wk after manufacture. Butters were subjected to the sensory analysis panel composed of 10 trained panelists. In single sessions, each panel member had to evaluate spreadability at 4°C, odor (total intensity, rancid, cream, milk, grass, hay, and hazelnut), flavor (total intensity, rancid, acidity, bitterness, cream, milk, grass, hazelnut, and metal), firmness and melting in the mouth giving a score between 0 and 10 (the more intense the criteria was, the greater the score). Spreadability consisted in scoring the ease to spread with a knife a homogeneous sample of butter at 4°C on a rusk.

### Statistical Analyses

Milk performance and intakes were determined using averaged data for the last 7 d of each period. Data were analyzed using the GLM procedure of SAS (SAS Institute, 1990) according to the following statistical model:

$$Y_{ijk} = \mu + C_i + D_j + P_k + e_{ijk}$$

where $Y_{ijk}$ = variable studied during the trial; $\mu$ = the overall mean of the population; $C_i$ = the effect due to the cow i for individual parameters or effect due to the group for group parameters; $D_j$ = the effect due to the treatment j (G0, G30, G60, G100); $P_k$ = the effect due to the period k; and $e_{ijk}$ = error associated with each $Y_{ijk}$.

The linear, quadratic, and cubic effects of treatments were analyzed by orthogonal contrasts. Results were expressed as least squares means with standards of the means. The significance threshold was set at $P \leq 0.05$ and the trend at $P \leq 0.10$.

## RESULTS

### Intake and Milk Production

Total intakes, energy, and PDI intakes were similar irrespective of the proportion of fresh grass in the diet (Table 2). Grass intake represented, as expected, 0, 33, 63, and 100% of forage intake for G0, G30, G60, and G100, respectively. Energy and PDI balances were not affected by treatment.

Milk yield increased linearly as the proportion of fresh grass in the diet increased (+0.21 kg/d per 10% of grass in the diet; Table 3).Curvilinear increases in milk protein yield and in protein content were observed. These increases were significant between G0 and G30 (+85 g/d and +0.17% for protein yield and protein content, respectively). Milk fat yield was not modified by

**Table 2.** Relationship between the proportion of fresh grass in the diet in replacement of corn silage and intake and balances

| | Diet[1] | | | | | Effect[3] | | |
|---|---|---|---|---|---|---|---|---|
| | G0 | G30 | G60 | G100 | RSD[2] | L | Q | C |
| Intake | | | | | | | | |
| Total, kg/d of DM | 18.6 | 18.0 | 18.6 | 18.1 | 0.82 | NS | NS | NS |
| Corn silage, kg/d of DM | 15.4 | 10.0 | 5.6 | 0 | 1.04 | *** | NS | NS |
| Grass, kg/d of DM | 0 | 4.7 | 9.7 | 14.9 | 0.79 | *** | NS | NS |
| Soybean, kg/d of DM | 2.9 | 2.0 | 1.0 | 0.0 | 0.01 | *** | *** | *** |
| Concentrate, kg/d of DM | 0.0 | 1.0 | 2.0 | 2.9 | 0.01 | *** | *** | *** |
| 5-18-10 minerals, kg/d of DM | 0.3 | 0.3 | 0.3 | 0.3 | | | | |
| Energy, $NE_l$/d | 30.0 | 29.6 | 31.1 | 30.8 | 1.30 | NS | NS | NS |
| PDIE,[4] g/d | 1,790 | 1,790 | 1,855 | 1,846 | 71.3 | NS | NS | NS |
| PDIN,[5] g/d | 1,645 | 1,790 | 1,958 | 2,092 | 90.3 | *** | NS | NS |
| Balances | | | | | | | | |
| Energy, $NE_l$/d | 2.6 | 1.0 | 2.6 | 2.4 | 1.18 | NS | NS | * |
| PDI,[6] g/d | 121 | 129 | 162 | 163 | 51.1 | NS | NS | NS |

[1]Diets: G0 = offered forage composed of 0% DM of grass and 100% DM of corn silage, G30 = offered forage composed of 30% DM of grass and 70% DM of corn silage, G60 = offered forage composed of 60% DM of grass and 40% DM of corn silage, and G100 = offered forage composed of 100% DM of grass and 0% DM of corn silage.

[2]RSD = Residual standard deviation of the ANOVA.

[3]Linear (L), quadratic (Q), and cubic (C) effects.

[4]PDIE = Digestible protein in the small intestine supplied by microbial protein from rumen-fermented OM (INRA, 1989).

[5]PDIN = Digestible protein in the small intestine supplied by microbial protein from rumen-degraded OM (INRA, 1989).

[6]Difference between the digestible proteins in the intestine (PDI) intakes and the PDI requirements.

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

diet but milk fat content tended to decrease linearly between G0 and G100.

### *Structural and Chemical Properties of Milk Fat*

A threshold value of the mode was attained with the G30 diet, compared with the G0 diet, and the response to the proportion of fresh grass tended to be quadratic (–0.31 μm from 30% of grass in the diet, $P = 0.072$; Table 3). Grass tended to induce a quadratic reduction in MFG average diameters, and a quadratic increase in the specific area of fat globule membrane surface, and in the surface of membrane synthesized daily (Table 3).

Correlations between FA contents in milk and in butter were significant and higher than 0.8, except for a few fatty acids (C14:1, C20:0, and C22:0) for which correlations were significant but lower than 0.8. Consequently, we have chosen to only present results for milk fat composition. Almost all the FA had a significant linear evolution with the proportion of fresh grass in the diet (Table 4).

Grass induced a linear increase in mono- and polyunsaturated fatty acid percentages at the expense of saturated fatty acid (**SFA**) percentage. The greatest percentage decrease was seen for C16:0 when the proportion of fresh grass in the diet increased (–6.9 points between G0 and G100). Short-chain even SFA (C4:0 to C14:0) also decreased linearly but not to the same extent as C16:0. Percentages of odd SFA (C11:0 to C17:0) increased linearly with the proportion of fresh grass in the diet.

Among monounsaturated fatty acid (**MUFA**), the proportion of C18:1 *trans*-11 greatly increased from 0.85 to 4.20%; C18:1 *cis*-9 (+*trans*-13) was generally not modified by diet. Grass induced a linear increase in others *trans* MUFA (C18:1 *trans*-6, *trans*-7, *trans*-8, and *trans*-15), except C18:1 *trans*-9 and *trans*-10. Grass induced a linear increase in *cis* MUFA (C18:1 *cis*-13 and *cis*-15). Monounsaturated FA with less than 18 carbons (C10:1, C14:1 *cis*-9, and C16:1 *cis*-9) decreased linearly or tended to decrease linearly with the proportion of fresh grass in the diet, except for C16:1 *cis*-7.

Between G0 and G100, polyunsaturated fatty acid (**PUFA**) percentages increased linearly because of the increase in C18:2 n-6 *trans trans* from 0.25 to 0.61%, C18:2 *cis*-9,*trans*-11 from 0.48 to 1.65%, and C18:3n-3 from 0.22 to 0.70%. On the other hand, C18:2 n-6 *cis cis* tended to decrease linearly. Very long chain PUFA (C20 and C22) were not modified by treatments.

The C18:1/C18:0 ratio increased linearly with the proportion of fresh grass in the diet, whereas C16:1/C16:0 and C14:1/C14:0 ratios were not modified by treatments. Atherogenicity index (Ulbricht and Southgate, 1991) decreased linearly. The spreadability index

**Table 3.** Relationship between the proportion of fresh grass in the diet in replacement of corn silage and milk, fat and protein yields, fat and protein contents and milk fat globule size

| | Diet[1] | | | | | Effect[3] | | |
|---|---|---|---|---|---|---|---|---|
| | G0 | G30 | G60 | G100 | RSD[2] | L | Q | C |
| Yield, kg/d | | | | | | | | |
| Milk | 23.7 | 24.8 | 26.1 | 25.8 | 1.75 | * | NS | NS |
| Fat | 0.99 | 1.03 | 1.03 | 1.01 | 0.094 | NS | NS | NS |
| Protein | 0.71 | 0.79 | 0.81 | 0.81 | 0.050 | *** | * | NS |
| Content, % | | | | | | | | |
| Fat | 4.28 | 4.39 | 4.09 | 4.01 | 0.319 | † | NS | NS |
| Protein | 3.11 | 3.28 | 3.22 | 3.23 | 0.100 | † | * | † |
| Fat globule size[4] | | | | | | | | |
| Mode, μm | 4.03 | 3.72 | 3.77 | 3.77 | 0.218 | † | † | NS |
| $d_{4,3}$, μm | 4.14 | 3.85 | 3.91 | 3.91 | 0.220 | NS | † | NS |
| $d_{3,2}$, μm | 3.50 | 3.28 | 3.31 | 3.31 | 0.174 | NS | † | NS |
| Specific area,[5] $m^2$/g of fat | 1.87 | 2.00 | 1.99 | 1.98 | 0.102 | NS | † | NS |
| Membrane yield, $m^2$/d | 1,844 | 2,051 | 2,053 | 2,015 | 197.4 | NS | NS | NS |

[1]Diets: G0 = offered forage composed of 0% DM of grass and 100% DM of corn silage, G30 = offered forage composed of 30% DM of grass and 70% DM of corn silage, G60 = offered forage composed of 60% DM of grass and 40% DM of corn silage, and G100 = offered forage composed of 100% DM of grass and 0% DM of corn silage.

[2]RSD = Residual standard deviation of the ANOVA.

[3]Linear (L), quadratic (Q), and cubic (C) effects.

[4]Milk fat globule (MFG) diameter for which distribution attains a peak; $d_{4,3}$ = Sauter diameter; $d_{3,2}$ = volume-weighted diameter.

[5]Specific area of MFG membrane.

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

(ratio of the major SFA C16:0 on the sum of C18:1 fatty acids) also decreased between G0 and G100 from 1.41 to 0.86.

### *Physicochemical Properties of Butter and Butter Fat*

Dry matter and fat contents of butter were not significantly modified by diet, despite an increase of 29 g/kg in DM and a decrease of 28 g/kg in fat content between G0 and G100 diets (Table 5).

Among treatments, the response to diet was similar at 5 and 10 mm of penetration. At 18 mm of penetration, forces for G0 and G60 were similar, weaker than G30, and stronger than G100. Forces tended to decrease between G30 and G100 inducing a quadratic relation between the proportion of fresh grass in the diet and butter hardness (Table 5).

Crystallization of butter fats began at similar T onset temperatures irrespective of the treatment applied (Figure 1 and Table 6). Cooling profiles were characterized by 2 exothermic peaks (i.e., crystallization peaks). The first peak, between 12 and 18°C, was the minor peak in energy (above all for G0). The second peak recorded on cooling was the major peak in energy. There was a linear shift of the curve toward the lower temperatures when the proportion of fresh grass in the diet increased (Figure 1 and Table 6). The increasing part of the second peak was not different between all anhydrous butter fats. It seemed that cooling enthalpy decreased as the proportion of fresh grass in the diet increased.

Melting profiles (Figure 2) were characterized by 3 endothermic peaks (i.e., melting peaks): one between −30 and 7°C (LMF), one between 7 and 26°C (MMF), and one between 26 and 45°C (high melting point fraction, **HMF**). In energy, these peaks could be classified thus: MMF > HMF > LMF. In regard to Figures 1 and 2, the effect of grass feeding on melting profiles was not as great as for cooling profiles. Between G0 and G100, the T offset tended to decrease linearly (Table 6). The LMF peak was similar for all treatments although enthalpy of fusion seemed to be higher for G0. There was a linear shift toward the lower temperatures of the MMF and HMF peaks when the proportion of fresh grass in the diet increased (Figure 2 and Table 6). The enthalpy of melting for the MMF peak seemed to decrease as the proportion of fresh grass in the diet increased.

Except at 4°C, solid fat content in anhydrous butter fat decreased curvilinearly when the proportion of fresh grass in the diet increased (Table 6). Treatments G0 and G60 were characterized by similar solid fat contents irrespective of temperature. Between G30 and G100, solid fat content seemed to decrease linearly with the proportion of fresh grass in the diet.

Panelists did not find any difference of spreadability between diets. Melting score increased and firmness in mouth decreased once the proportion of fresh grass in

**Table 4.** Relationship between the proportion of fresh grass in the diet in replacement of corn silage and milk fatty acid composition in milk

| Fatty acid (FA), g/100 g of total FA | Diet[1] | | | | | Effect[3] | | |
|---|---|---|---|---|---|---|---|---|
| | G0 | G30 | G60 | G100 | RSD[2] | L | Q | C |
| C4:0 | 4.3 | 3.8 | 3.9 | 3.9 | 0.26 | * | * | NS |
| C6:0 | 2.5 | 2.3 | 2.4 | 2.2 | 0.10 | *** | NS | * |
| C8:0 | 1.5 | 1.4 | 1.5 | 1.3 | 0.09 | † | NS | † |
| C10:0 | 3.3 | 3.4 | 3.5 | 3.1 | 0.27 | NS | * | NS |
| C10:1 | 0.27 | 0.26 | 0.26 | 0.24 | 0.023 | † | NS | NS |
| C11:0 | 0.03 | 0.05 | 0.07 | 0.05 | 0.019 | † | * | NS |
| C12:0 | 3.7 | 3.8 | 3.9 | 3.4 | 0.32 | NS | * | NS |
| C13:0 | 0.09 | 0.12 | 0.12 | 0.14 | 0.028 | * | NS | NS |
| C14:0 | 11.7 | 11.6 | 11.8 | 10.8 | 0.43 | ** | * | NS |
| C14:0 anteiso | 0.12 | 0.09 | 0.09 | 0.11 | 0.026 | NS | * | NS |
| C14:1 *cis*-9 | 0.87 | 0.86 | 0.89 | 0.83 | 0.081 | NS | NS | NS |
| C15:0 | 0.77 | 0.99 | 1.10 | 1.30 | 0.109 | *** | NS | NS |
| C15:0 iso | 0.26 | 0.28 | 0.31 | 0.35 | 0.031 | *** | NS | NS |
| C15:0 anteiso | 0.45 | 0.49 | 0.58 | 0.66 | 0.042 | *** | NS | NS |
| C16:0 | 31.0 | 28.4 | 26.8 | 24.1 | 1.85 | *** | NS | NS |
| C16:0 iso | 0.31 | 0.26 | 0.25 | 0.26 | 0.048 | † | NS | NS |
| C16:1 *cis*-9 | 1.73 | 1.61 | 1.54 | 1.46 | 0.134 | ** | NS | NS |
| C16:1 *cis*-7 | 0.19 | 0.20 | 0.20 | 0.22 | 0.024 | * | NS | NS |
| C17:0 | 0.46 | 0.52 | 0.55 | 0.64 | 0.035 | *** | NS | NS |
| C17:0 iso | 0.37 | 0.45 | 0.48 | 0.57 | 0.043 | *** | NS | NS |
| C17:0 anteiso | 0.42 | 0.43 | 0.46 | 0.46 | 0.021 | ** | NS | NS |
| C18:0 | 10.3 | 11.1 | 10.5 | 11.2 | 0.97 | NS | NS | † |
| C18:1 *cis*-15 | 0.22 | 0.35 | 0.41 | 0.56 | 0.146 | ** | NS | NS |
| C18:1 *trans*-15 | 0.14 | 0.26 | 0.24 | 0.34 | 0.102 | ** | NS | NS |
| C18:1 *cis*-13 | 0.08 | 0.12 | 0.17 | 0.20 | 0.030 | *** | NS | NS |
| C18:1 *cis*-11 | 0.51 | 0.48 | 0.48 | 0.44 | 0.073 | NS | NS | NS |
| C18:1 *trans*-11 | 0.85 | 1.45 | 3.12 | 4.70 | 0.768 | *** | † | NS |
| C18:1 *trans*-10 | 0.79 | 0.59 | 0.54 | 0.27 | 0.216 | ** | NS | NS |
| C18:1 *trans*-9 | 0.31 | 0.25 | 0.07 | 0.07 | 0.160 | ** | NS | NS |
| C18:1 *cis*-9 + *trans*-13 | 19.4 | 20.4 | 19.4 | 21.1 | 1.26 | † | NS | * |
| C18:1 *trans*-6 + *trans*-7 + *trans*-8 | 0.29 | 0.35 | 0.49 | 0.47 | 0.164 | * | NS | NS |
| C18:2 n-6 *cis cis* | 1.55 | 1.33 | 1.35 | 1.26 | 0.271 | NS | NS | NS |
| C18:2 n-6 *trans trans* | 0.25 | 0.40 | 0.49 | 0.61 | 0.074 | *** | NS | NS |
| C18:2 CLA *cis*-9, *trans*-11 | 0.48 | 0.54 | 1.21 | 1.65 | 0.216 | *** | * | * |
| C18:3 n-3 | 0.22 | 0.40 | 0.56 | 0.70 | 0.043 | *** | NS | NS |
| C20:0 | 0.14 | 0.16 | 0.04 | 0.02 | 0.054 | *** | NS | * |
| C20:3 n-6 | 0.10 | 0.11 | 0.09 | 0.12 | 0.068 | NS | NS | NS |
| C20:4 n-6 | 0.10 | 0.10 | 0.10 | 0.10 | 0.027 | NS | NS | NS |
| C22:0 | 0.026 | 0.031 | 0.033 | 0.058 | 0.0341 | NS | NS | NS |
| C22:5 n-3 | 0.067 | 0.029 | 0.036 | 0.026 | 0.0315 | † | NS | NS |
| C22:5 n-6 | 0.012 | 0.013 | 0.006 | 0.036 | 0.0223 | NS | † | NS |
| Short-chain FA (C4 to C14) | 28.4 | 27.9 | 28.5 | 26.2 | 1.16 | * | † | * |
| Medium-chain FA (C15 to C17) | 36.0 | 33.6 | 32.3 | 30.1 | 1.70 | *** | NS | NS |
| Long-chain FA (C18 to C22) | 36.1 | 38.8 | 39.6 | 44.1 | 2.19 | *** | NS | NS |
| Saturated FA | 71.8 | 69.8 | 68.4 | 64.7 | 1.50 | *** | NS | NS |
| Monounsaturated FA | 25.9 | 27.5 | 28.1 | 31.2 | 1.39 | *** | NS | NS |
| Polyunsaturated FA | 2.81 | 2.94 | 3.87 | 4.52 | 0.382 | *** | † | † |
| Unpaired FA | 2.8 | 3.3 | 3.7 | 4.2 | 0.27 | *** | NS | NS |
| C18:1/C18:0 | 2.27 | 2.21 | 2.44 | 2.57 | 0.183 | ** | NS | NS |
| C14:1/C14:0 | 0.072 | 0.071 | 0.074 | 0.074 | 0.0055 | NS | NS | NS |
| C16:1/C16:0 | 0.064 | 0.064 | 0.064 | 0.069 | 0.0064 | NS | NS | NS |
| Atherogenicity index[4] | 2.93 | 2.69 | 2.48 | 2.01 | 0.211 | *** | NS | NS |
| C16:0/C18:1 | 1.41 | 1.21 | 1.09 | 0.86 | 0.135 | *** | NS | NS |

[1]Diets: G0 = offered forage composed of 0% DM of grass and 100% DM of corn silage, G30 = offered forage composed of 30% DM of grass and 70% DM of corn silage, G60 = offered forage composed of 60% DM of grass and 40% DM of corn silage, and G100 = offered forage composed of 100% DM of grass and 0% DM of corn silage.

[2]RSD = Residual standard deviation of the ANOVA.

[3]Linear (L), quadratic (Q), and cubic (C) effects.

[4]Atherogenicity index = sum of lauric (C12:0), palmitic (C16:0), and 4 times myristic acid (C14:0) contents divided by the unsaturated FA content.

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

**Table 5.** Relationship between the proportion of fresh grass in the diet in replacement of corn silage and butter characteristics (composition and rheological parameters)

| | Diet[1] | | | | | Effect[3] | | |
|---|---|---|---|---|---|---|---|---|
| | G0 | G30 | G60 | G100 | RSD[2] | L | Q | C |
| DM, % | 87.1 | 85.9 | 85.6 | 84.2 | 1.7 | NS | NS | NS |
| Fat content, % | 85.5 | 84.6 | 84.1 | 82.7 | 1.6 | NS | NS | NS |
| Rheology | | | | | | | | |
| Force at 5 mm, N | 22 | 26 | 23 | 20 | 4.2 | NS | NS | NS |
| Force at 10 mm, N | 81 | 93 | 77 | 65 | 10.9 | NS | † | NS |
| Force at 18 mm, N | 208 | 245 | 209 | 182 | 19.5 | NS | * | NS |

[1]Diets: G0 = offered forage composed of 0% DM of grass and 100% DM of corn silage, G30 = offered forage composed of 30% DM of grass and 70% DM of corn silage, G60 = offered forage composed of 60% DM of grass and 40% DM of corn silage, and G100 = offered forage composed of 100% DM of grass and 0% DM of corn silage.

[2]RSD = Residual standard deviation of the ANOVA.

[3]Linear (L), quadratic (Q), and cubic (C) effects.

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

the diet reached 30%; responses to the proportion of fresh grass were curvilinear for these 2 scores (Table 7).

Grass induced a curvilinear decrease in the perception of the total intensity of the odor (from 5.8 to 3.4), correlated to a linear decrease in the perception of the rancid odor (from 3.1 to 0.0). The total intensity of the flavor was more intense for G0 compared with the other diets and the response was curvilinear. This scoring was correlated to a curvilinear decrease in the perception of the rancid flavor. Diets did not modify the perception of the others flavors (acidity, bitterness, cream, milk, hazelnut, and metal) and odors (cream, milk, grass, hay, and hazelnut) of butter.

### *Correlations Between Variables*

Melting and firmness scores were correlated to solid fat content determined by DSC at 37°C (R = –0.86 and

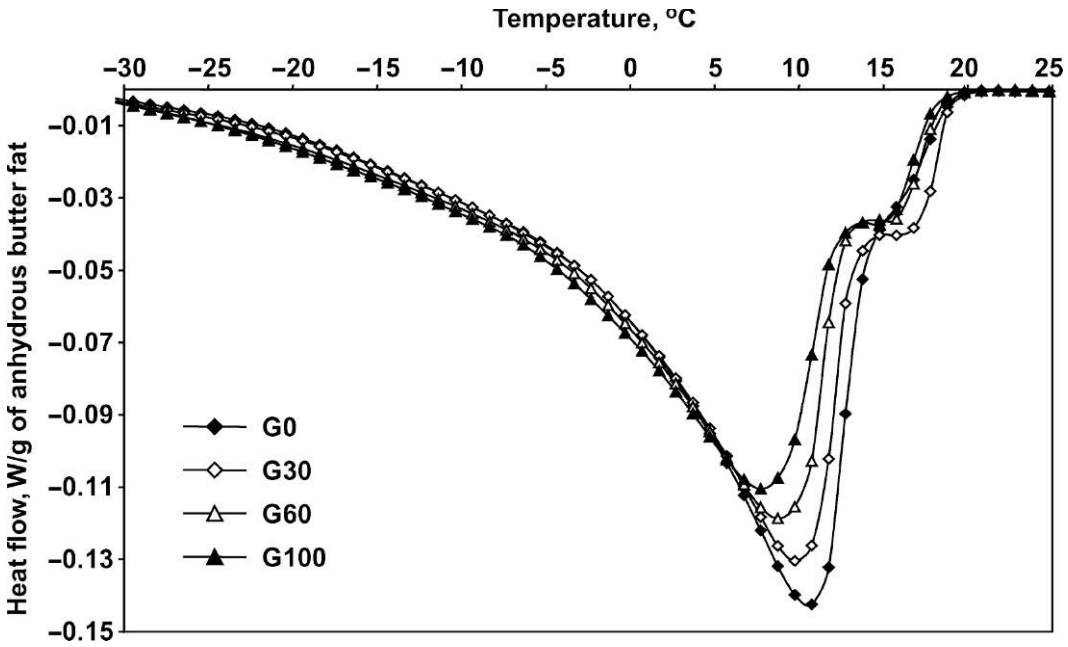


**Figure 1.** Relationship between the proportion of fresh grass in the diet in replacement of corn silage and thermal behavior of anhydrous butter fat during crystallization recorded by differential scanning calorimetry during cooling at 2°C/min from 60°C to –40°C. Diets: G0 = offered forage composed of 0% DM of grass and 100% DM of corn silage, G30 = offered forage composed of 30% DM of grass and 70% DM of corn silage, G60 = offered forage composed of 60% DM of grass and 30% DM of corn silage, and G100 = offered forage composed of 100% DM of grass and 0% DM of corn silage.

**Table 6.** Relationship between the proportion of fresh grass in the diet in replacement of corn silage and crystallization and final melting temperature of anhydrous butter fat and solid fat contents at different temperatures determined by differential scanning calorimetry (DSC)

| | Diet[1] | | | | | Effect[3] | | |
|---|---|---|---|---|---|---|---|---|
| | G0 | G30 | G60 | G100 | RSD[2] | L | Q | C |
| Temperature,[4] °C | | | | | | | | |
| T onset | 18.8 | 19.0 | 18.5 | 18.5 | 0.60 | NS | NS | NS |
| First peak crystallization | 16.0 | 16.1 | 15.9 | 15.2 | 0.54 | NS | NS | NS |
| Second peak crystallization | 10.6 | 9.6 | 8.9 | 8.3 | 0.49 | * | NS | NS |
| T offset LMF peak | 1.9 | 3.1 | 3.3 | 4.0 | 1.12 | NS | NS | NS |
| T offset MMF peak | 16.9 | 17.3 | 16.4 | 16.3 | 0.59 | NS | NS | NS |
| T offset | 39.7 | 40.0 | 39.3 | 39.1 | 0.23 | † | NS | NS |
| Solid fat content,[5] % | | | | | | | | |
| 4°C | 82.0 | 84.2 | 83.2 | 81.7 | 0.29 | NS | ** | † |
| 8°C | 78.5 | 81.0 | 79.3 | 77.2 | 0.18 | * | ** | * |
| 12°C | 71.4 | 74.0 | 71.6 | 68.4 | 0.18 | ** | ** | ** |
| 18°C | 46.1 | 49.2 | 46.5 | 43.8 | 0.21 | ** | ** | ** |
| 27°C | 19.8 | 22.3 | 20.8 | 19.5 | 0.16 | * | ** | ** |
| 37°C | 1.6 | 1.8 | 1.3 | 1.0 | 0.04 | ** | ** | ** |

[1]Diets: G0 = offered forage composed of 0% DM of grass and 100% DM of corn silage, G30 = offered forage composed of 30% DM of grass and 70% DM of corn silage, G60 = offered forage composed of 60% DM of grass and 40% DM of corn silage, and G100 = offered forage composed of 100% DM of grass and 0% DM of corn silage.

[2]RSD = Residual standard deviation of the ANOVA.

[3]Linear (L), quadratic (Q), and cubic (C) effects.

[4]T onset (beginning crystallization temperature) of anhydrous butter fat determined from the cooling of the samples at 2°C/min from 60°C to –40°C; T offset LMF, T offset MMF, and T offset = T offset (final melting temperature) of the low melting fraction (LMF), medium melting fraction (MMF), and anhydrous butter fat, respectively, determined from the heating of the samples at 2°C/min from –40°C to 60°C after cooling at 2°C/min.

[5]Solid fat content of anhydrous butter fat determined by calculating the cumulative area under the melting curve recorded by DSC during heating of the samples at 2°C/min from –40°C to 60°C after cooling at 2°C/min.

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

R = 0.81, respectively), to DM percentage (R = –0.53 and R = 0.55, respectively), to MUFA (R = 0.59 and R = –0.60, respectively), to PUFA (R = 0.78 and R = –0.71, respectively), and to C16:0/C18:1 ratio (R = –0.74 and R = 0.75, respectively). The correlation matrix also indicated that solid fat content (at 37°C) and rheology (force at 10 mm) were correlated to MUFA content (R = –0.47 and R = –0.67, respectively), to PUFA content (R = –0.76 and R = –0.64, respectively), to C16:0/C18:1 ratio (R = 0.67 and R = 0.66, respectively), and to DM content (R = 0.45 and R = 0.66, respectively).

## DISCUSSION

### *Animal Performance*

In our study, energy and protein intakes were similar between diets and energy and protein balances were over target for all the diets. Compared with literature reports (Dhiman et al., 1999; Agenäs et al., 2002; Hurtaud et al., 2002a; Schroeder et al., 2003), the effects observed in this study were only attributable to the proportion of fresh grass in the diet and not to energy or protein deficit, or interaction induced by the proportion of fresh grass in the diet.

In these experimental conditions, we observed a strong linear increase in milk and protein yields and in protein content in disagreement with published reports (Dhiman et al., 1999; Agenäs et al., 2002; Schroeder et al., 2003). In our study, these increases should be due to a specific effect of grass. Indeed, because grass induced a linear increase in the propionic acid content in the rumen (+5.8 points between G0 and G100, data not shown), the increase in milk and protein productions, and thus protein content, could be due to a modification of the nature of the energy provided to the udder, as shown by Rigout et al. (2003). The lack of effect of increasing the proportion of fresh grass in the diet on the production of fat is in disagreement with the literature, which highlights a decrease in fat production (Dhiman et al., 1999; Agenäs et al., 2002; Schroeder et al., 2003). However, because of its positive effect on milk yield, grass induced a linear decrease in fat content according to its proportion in the diet by dilution of the same quantity of fat in a greater volume of milk. This decrease in fat content is consistent with the previously

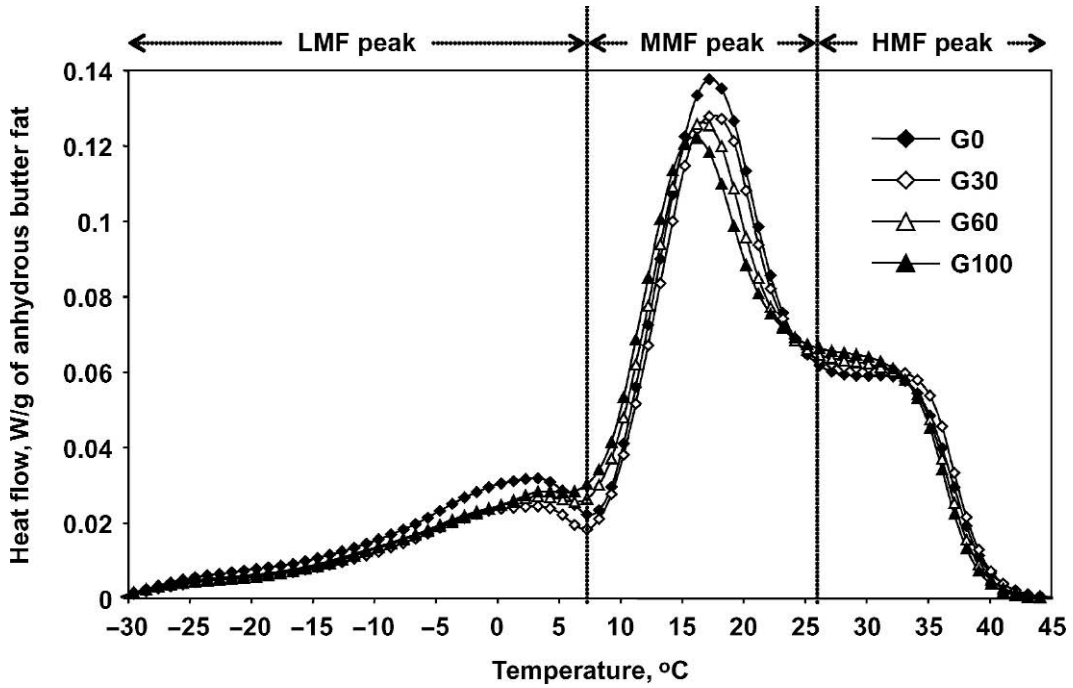


**Figure 2.** Relationship between the proportion of fresh grass in the diet in replacement of corn silage and thermal behavior of anhydrous butter fat during melting recorded by differential scanning calorimetry during heating at 2°C/min from –40°C to 60°C after cooling at 2°C/min. Diets: G0 = offered forage composed of 0% DM of grass and 100% DM of corn silage, G30 = offered forage composed of 30% DM of grass and 70% DM of corn silage, G60 = offered forage composed of 60% DM of grass and 30% DM of corn silage, and G100 = offered forage composed of 100% DM of grass and 0% DM of corn silage. LMF = Low melting fraction; MMF = medium melting fraction; HMF = high melting fraction.

cited research and may be explained by an inhibiting effect of the long-chain UFA on the de novo synthesis (Baumann and Griinari, 2001).

### *Physicochemical Characteristics of Milk Fat*

***MFG Size.*** Our results showed that MFG size decreased when the proportion of fresh grass in the diet increased, which is in agreement with Mulder and Walstra (1974). Furthermore, we showed that the effect of grass on MFG size was maximized when its proportion in the diet reached 30%. Hurtaud et al. (2002a) showed that the transition from winter diet to pasture (pasture during the day, corn silage at night) induces no decrease and that only full grazing induces a significant reduction of 0.3 μm in the MFG diameter. In our study, a 1-wk transition period was carried out before the week of measurements allowing an adaptation of the digestion and synthesis activities. This adaptation period did not exist in the study of Hurtaud et al. (2002a) in which measurements were carried out when transition began. The maximum reduction in MFG size would thus seem to be reached when the proportion of fresh grass in the diet is 30% after a period of few days of adaptation. Decreases in the mode, $d_{4,3}$, and $d_{3,2}$, and an increase in the specific surface MFG with grass would be related to a specific effect of grass, irrespective of its offered form. Indeed, compared with a corn silage-based diet, decrease in MFG size is 0.21 μm at pasture (Hurtaud et al., 2002a), 0.27 μm with grass silage (Couvreur et al., 2004b), 0.19 μm (Ingr et al., 1972) and 0.28 μm (Couvreur et al., 2004a) with haylage, and 0.38 μm with hay (Hurtaud et al., 2002b).

As shown by Wiking et al. (2004), the decrease in MFG size can be explained by its positive correlation with fat content. Nevertheless, the mechanisms responsible for this decrease have not yet been highlighted. Many authors have suggested that the mechanisms responsible for this decrease in fat globule size due to grass in the diet could be of cellular origin implying the synthesis and secretion activities (Ingr et al., 1972; Briard et al., 2003; Wiking et al., 2004). By showing a positive correlation between MFG size and C16:0, C18:0, and C18:1 percentages, these authors have suggested that, jointly with the synthesis activity, the secretion activity of milk fat could be modified by incorporation of grass in the diet. To our knowledge, few studies have determined which step of secretion could be responsible for reduction in the MFG size. Briard et al. (2003) supposed that membrane synthesis is limited by

**Table 7.** Relationship between the proportion of fresh grass in the diet in replacement of corn silage and butter characteristics determined by sensory analysis

| | Diet[1] | | | | | Effect[3] | | |
|---|---|---|---|---|---|---|---|---|
| | G0 | G30 | G60 | G100 | RSD[2] | L | Q | C |
| Spreadability[4] | 4.2 | 4.3 | 3.4 | 4.3 | 2.27 | NS | NS | NS |
| Melting in mouth | 5.0 | 4.9 | 6.3 | 7.2 | 1.59 | *** | † | NS |
| Firmness in mouth | 3.3 | 3.6 | 2.2 | 1.4 | 1.07 | *** | ** | ** |
| Odor | | | | | | | | |
| Total intensity | 5.8 | 4.5 | 3.8 | 3.4 | 1.21 | *** | * | NS |
| Rancid | 3.1 | 1.3 | 0.9 | 0.0 | 1.80 | *** | NS | NS |
| Cream | 1.5 | 2.0 | 2.0 | 1.9 | 1.00 | NS | NS | NS |
| Milk | 1.3 | 1.6 | 1.4 | 1.4 | 0.93 | NS | NS | NS |
| Grass | 0.1 | 0.2 | 0.2 | 0.2 | 0.37 | NS | NS | NS |
| Hay | 0.00 | 0.04 | 0.02 | 0.04 | 0.150 | NS | NS | NS |
| Hazelnut | 0.07 | 0.10 | 0.04 | 0.02 | 0.188 | NS | NS | NS |
| Flavor | | | | | | | | |
| Total intensity | 4.8 | 3.6 | 3.6 | 3.7 | 1.07 | ** | ** | NS |
| Rancid | 2.6 | 0.8 | 0.6 | 0.2 | 1.42 | *** | ** | † |
| Acid | 0.4 | 0.2 | 0.4 | 0.3 | 0.47 | NS | NS | † |
| Bitter | 0.00 | 0.00 | 0.03 | 0.08 | 0.167 | NS | NS | NS |
| Cream | 1.2 | 1.6 | 1.4 | 1.3 | 0.85 | NS | † | NS |
| Milk | 1.3 | 1.6 | 1.4 | 1.6 | 0.93 | NS | NS | NS |
| Grass | 0.00 | 0.04 | 0.00 | 0.03 | 0.126 | NS | NS | NS |
| Hazelnut | 0.12 | 0.16 | 0.07 | 0.02 | 0.220 | † | NS | NS |
| Metal | 0.24 | 0.07 | 0.11 | 0.08 | 0.401 | NS | NS | NS |

[1]Diets: G0 = offered forage composed of 0% DM of grass and 100% DM of corn silage, G30 = offered forage composed of 30% DM of grass and 70% DM of corn silage, G60 = offered forage composed of 60% DM of grass and 40% DM of corn silage, and G100 = offered forage composed of 100% DM of grass and 0% DM of corn silage.

[2]RSD = Residual standard deviation of the ANOVA.

[3]Linear (L), quadratic (Q), and cubic (C) effects.

[4]Score between 1 and 10 to evaluate the ease of spreading with a knife a homogeneous sample of butter at 4°C on a rusk.

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

the quantity of UFA in the cell. The increase in UFA with the proportion of fresh grass observed in our study could explain the increase in membrane production and the secretion of a similar quantity of fat into smaller globules. However, Wiking et al. (2004) have suggested that the mammary epithelial cell has a limited capacity for apical membrane synthesis. The limited quantity of apical membrane would impose a minimum average MFG diameter of secretion, explaining the threshold effect observed.

***FA Composition.*** The modification of the FA profile between the extreme diets (G0 and G100) agrees with the literature (Cullinane et al., 1984; Kelly et al., 1998; Chilliard et al., 2001; Hurtaud et al., 2002a; Loor et al., 2003; Schroeder et al., 2003). Compared with these studies, our results highlighted a linear evolution of the fatty acid composition when the proportion of fresh grass increased in the diet. Agabriel et al. (2004) also observed that dairy systems using a mixed forage composed primarily of grass (60% grazed grass plus 30% corn silage) resulted in an intermediate FA profile, compared with dairy systems using a mixed forage composed in majority by corn silage (60% corn silage plus 40% grass silage) and dairy systems using a full grazing diet.

Rye grass and clover contain between 1 and 3% of fat comprising mainly C18:3 (Givens et al., 2000) whereas fat contained in corn silage is mainly C18:2. In our study, a linear increase in C18:3 intake and a linear decrease of C18:2 intake when the proportion of fresh grass increased could be supposed. This linear increase in C18:3 intake could explain the linear increase in C18:3 percentage in milk because C18:3 in milk only comes from the C18:3 nonhydrogenated in the rumen, and the hydrogenation level of C18:3 is quite similar between diets. This increase in C18:3 intake also induced an increase in the ruminal bypass of its hydrogenation intermediates (C18:1 *trans*-11 and C18:0) and in their availability within the mammary epithelial cell (Sauvant and Bas, 2001). Thus, at a same desaturation activity, which could be supposed because C16:1/C16:0 and C14:1/C14:0 ratios were similar between diets, the linear increase in the C18:3, C18:1 *trans*-11, and C18:0 availabilities within the mammary epithelial cell implied a linear increase in C18:3, C18:2 *cis*-9,*trans*-11, C18:1 *cis*-9, C18:1 *trans*-11, and C18:0

percentages in milk. Similar increases with grass in the diet in replacement of alfalfa hay were highlighted by Dhiman et al. (1999). Fatty acids containing more than 18 carbons are powerful inhibitors of de novo fatty acid synthesis. Therefore, the linear increase in their availability within the mammary epithelial cell induced a decrease in the synthesis activity and in the short- and medium-chain FA contents.

In accordance to these results and compared with Chilliard et al. (2001), we showed that increasing the proportion of fresh grass in the diet induced a linear improvement of the nutritional value of milk fat. Fatty acid C18:1 *trans*-11, and *trans* FA in general, increased (2.85, 3.50, 5.14, and 6.62% for G0, G30, G60, and G100, respectively; $P < 0.001$) with the proportion of fresh grass. The increase in the *trans* FA content is associated with a significant decrease in SFA, the latter being associated with nutritional benefits. On the other hand, new regulations about the labeling of *trans* FA, associated with a carcinogenic value, for fatty products (including those from ruminants) in the United States. However, other countries such as Denmark exclude the "natural" *trans* fatty acids from labeling. Consequently, the impact of the present data on butter consumption may be modulated by specific regulations, according to countries around the world. However, C18:1 *trans*-11 is the major precursor of conjugated linoleic acid, C18:2 *cis*-9,*trans*-11, which has a positive value from a nutritional point of view. In this context, we can suppose that the increase of conjugated linoleic acid may partially offset the impact of the increase in *trans* FA content on consumer behavior in the United States.

### Sensory and Functional Properties of Butter

***Sensory Properties.*** Rancidity of butters (flavor and odor) decreased when the proportion of fresh grass in the diet increased. The principal studies listed in the literature highlight a decrease in the rancidity character of butters produced with cows fed fresh grass compared with corn silage (Deeth and Fitz-Gerald, 1983). Rancid flavor and odor are due to the lipolysis of milk fat. The lipase, or lipolysis enzyme, catalyzes the triglycerides hydrolysis. It can be of endogenous origin (Deeth and Fitz-Gerald, 1983), or of microbial origin (i.e., introduced into milk during storage, transport, or manufacture). Regardless of its origin, by hydrolyzing triglycerides, it releases free FA. The shortest (in particular C4:0 and C6:0, in the case of butter) are mainly responsible for rancid character (Walstra et al., 1999). In our study, the process was similar for all treatments suggesting an identical lipolysis inducement irrespective of the diet applied. Thus, only the proportion of fresh grass in the diet could induce a variation of the rancid character of butter. Indeed, grass decreased linearly C4:0 content in milk and butter fat. With a similar lipolysis activity, the quantity of free C4:0 would have been reduced with the proportion of fresh grass in the diet inducing a decrease in the perception of butter rancidity. The positive correlation between C4:0 content in milk and butter fat and the scores of rancidity in sensory analysis would confirm this assumption.

***Rheological and Thermal Properties.*** Hardness of butter tended to decrease between G0 and G100, and was well correlated with the perception of firmness and melting in mouth by the panelists. These results agree with the study of Hurtaud et al. (2002a) even if these authors had observed a maximum improvement in firmness at the transition period from corn silage to pasture.

The modification of FA profile, and in particular, the decrease in C16:0/C18:1 ratio when the proportion of fresh grass in the diet increased from 0 to 100% could explain the improvement in rheological properties by the modification of the thermal behavior of butter fat and the reduction in solid fat content (Lavigne, 1995). A comparative study undertaken on industrial butters produced in winter and summer in France (n = 480) agrees with this assumption (Guyonnet, 1989). Guyonnet characterized summer butters (pasture) by an average C16:0/C18:1 ratio of 1.00 and a share strength 45% lower, compared with winter butters (silage-based diets, C16:0/C18:1 = 1.50).

In our study, all treatments induced 2 crystallization peaks in DSC analysis, as previously observed (Lopez et al., 2005). The beginning of the second peak of crystallization started at a lower temperature and the enthalpy of crystallization of this peak decreased when the proportion of fresh grass in diet was increased. The strongest UFA contents in the samples, by inducing a greater number of defects in the triglyceride structures, facilitate the nucleation and crystallization at a lower energy cost (Lavigne, 1995). Moreover, the 3 peaks of melting corresponding to the 3 fractions of triglycerides (LMF, MMF, and HMF) were observed in accordance with studies in the literature (Timms, 1980). The decrease in the melting enthalpy of the MMF peak and in the final melting temperature with the increasing proportion of fresh grass can also be explained by the modifications of the crystalline structure formation and their stability to heating caused by triglycerides and thus FA compositions. Indeed, Lavigne (1995) showed that the MMF fraction would be correlated to the contents of C4:0-C16:0-C16:0 and C16:0-C16:0-C18:1 triglycerides, and to the C4:0/C18:1 and C16:0/C18:1 ratios. The HMF fraction would be correlated to the contents of C16:0, of C16:0-C16:0-C18:1 and C16:0-C16:0-C16:0 triglycerides. The linear increase in C18:1 con-

tent in our study might, by the linear increase in the palmitic acid-palmitic acid-oleic acid and palmitic acid-oleic acid-oleic acid contents, partly explain the linear reduction in MMF peak with the proportion of fresh grass. Moreover, the linear decrease in C16:0 content might, by the linear decrease in palmitic acid-palmitic acid-palmitic acid content, partly explain the linear decrease in the final melting temperature. Thus, the C16:0/C18:1 ratio would seem to be a good indicator of the crystallization state of butter fat and the hardness of butter. The modification of melting behavior induced a decrease in solid fat content with the proportion of fresh grass in the diet and logically decreased butter hardness at 4°C and increased melting in mouth (Lavigne, 1995).

In our study, the modifications in thermal properties, partly due to the decrease in C16:0/C18:1 ratio, were sufficient to induce an approximate 30% decrease in butter hardness and an improvement of 30% in melting in mouth between G0 and G100. However, even if 30% grass in the diet was sufficient to induce a noticeable improvement in the C16:0/C18:1 ratio and the thermal behavior of butter fat, this was not sufficient to induce an improvement in texture in mouth. The improvement in texture became noticeable between 30 and 60% of grass in the diet, but complementary studies should be carried out to determine this proportion more precisely.

Alternatively, the texture improvement may be also related to the increase in butter moisture when the proportion of fresh grass increases in the diet. This result agrees with the conclusion of Foley (1978) who showed that firmness is reduced by 50% when butter moisture increases from 0 to 40%. Although Brodin (1989) affirmed that small increases in moisture do not inevitably induce noticeable improvement in rheology, it remains impossible in our study to determine if the improvement in butter rheological properties is due to the increase in UFA contents, the increase in moisture, or both. On the other hand, the decrease in MFG size with grass incorporation would not seem sufficient to induce differences in butter hardness (Goudédranche et al., 2000).

## CONCLUSIONS

Our study demonstrated that inclusion of fresh grass in the cow diet, compared with corn silage, can modify the nutritional and sensorial properties of butter. It appeared that effects are mostly linear when the proportion of fresh grass in the forage was between 30 and 60%. The optimal proportion of grass in the diet (between 30 and 60%) remains to be specified. The reproducibility of the results when cows are at pasture (the offered proportion of fresh grass being obtained by increasing access times to the paddocks) also needs to be investigated. Finally, further studies are needed to detail the specific effects of grass (type of grass, vegetative stage) on the sensorial and nutritional properties of butter.

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