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Original article

Tanker milk variability in fatty acids according to farm feeding and husbandry practices in a French semi-mountain area

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Abstract – The objective of this study was to relate farm feeding practices in different production systems to milk fatty acid (FA) composition on the scale of round tankers. Milks from 10 collection rounds in the French department of the Haute-Loire (10 to 36 herds per collection round) were sampled twice and three times during winter and grazing periods, respectively. The collection rounds were principally characterised by the forage system (grass or maize silage). Nine variants of milk production conditions were defined: four for the winter feeding period (W1 to W4) and five for the grazing period (G1 to G5). Over the year rumenic acid was positively correlated with vaccenic acid (r = 0.99), all the other *trans* and *c*11 isomers of C18:1, oleic acid (r = 0.79), linolenic acid (r = 0.82) and eicosapentaenoic acid (C20:5*n*-3, EPA). The milk fat from cows grazed on grass had a higher proportion of total *trans* FA (including *trans* C18:1, non-conjugated C18:2 and *c*9*t*11-CLA) and total *cis* C18:1, and a lower proportion of medium-chain saturated FA (−9.50 g·100 g^{-1} for C16:0 between G5 vs. W1) and monounsaturated FA (mainly *c*9-C16:1) than that from grass silage-based (and concentrate-supplemented) diets. Also, *anteiso*-15, C18:0, *c*9-, *t*6+7+8-, *t*9-, *t*11- and *t*13+14-C18:1, *c*9*t*11-CLA (r = 0.65), *t*11*c*15-C18:2, C18:3*n*-3 (r = 0.68) and EPA (r = 0.64) were positively linked to permanent grassland forages (green or conserved) on the scale of the year. During winter, *trans* (*t*6+7+8, *t*10, *t*12 and *t*13+14) and *cis* (*c*12, *c*13 and *t*16+*c*14) isomers of C18:1 were positively correlated with the proportion of maize silage in the diet (r = 0.47 to 0.91). The wide range of milk FA composition from the rounds observed in this study was closely linked to the variants in feeding and husbandry conditions. Our data confirm the strong effect of nutritional factors on milk FA composition of tanker milk shown in experimental trials.

round tanker milk / farm feeding and husbandry practices / *trans*, conjugated and *n*-3 fatty acids / grassland / dairy cow

摘要 – 法国半山区域牧场奶牛饲养模式对牛奶脂肪酸的影响。本文主要研究了不同农场饲养体系下, 由贮奶罐保存的牛奶脂肪酸的变化。分别在法国上卢瓦尔省半山区域的 10 个牧场 (每个采样点设 10 ~ 36 个放牧群) 进行采样, 在冬季和放牧期分别采集牛奶样品 2 ~ 3 次。所有采样点的饲料主要是以草料为主 (草料和青贮玉米)。分别设定了 9 种奶牛的生产条件, 冬季饲养期间有四种 (W1 ~ W4), 放牧期间有5种 (G1 ~ G5)。经过一年的实验结果发现: 9 顺, 11 反-亚油酸与 11 反-十八碳烯酸 (r = 0.99)、其他所有的反式和 11 顺-十

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八碳烯酸的异构体、油酸 (r = 0.79)、亚麻酸 (r = 0.82) 和二十碳五稀酸 (EPA) 都呈正相关。以放 牧为主与以草料和青贮饲料 (补充了浓缩饲料) 为主的奶牛相比, 前者乳脂肪中总反式脂肪酸 (包括反式 C18:1 、非共轭 C18:2 和 *c*9*t*11-共轭亚油酸) 和总顺式 C18:1 高于后者, 但是其中链饱和脂肪酸 (在 G5 与 W1 之间 C16:0 相差 9.50 g·100 g^{-1}) 和单不饱和脂肪酸 (主要是 *c*9-C16:1) 则低于后者。另外, *anteiso*-15、C18:0、*c*9-、*t*6+7+8-、*t*9-、*t*11-和 *t*-13+14-C18:1、*c*9*t*11-共轭亚油酸 (r = 0.65)、*t*11*c*15-C18:2、C18:3*n*-3 (r = 0.68) 和二十碳五烯酸 (r = 0.64), 这些脂肪酸的含量具与在永久草场中放牧的饲养方式 (青草或干草) 呈正相关。在冬季饲养期间, C18:1 的反式 (*t*6+7+8、*t*10、*t*12 和 *t*13+14) 和顺式 (*c*12、*c*13 和 *t*16+*c*14) 异构体也与饲料中青贮玉米饲料的比例呈正相关 (r = 0.47 ~ 0.91), 此项研究表明, 不同采样点存在较大的乳脂肪酸组成变化范围与奶牛的饲养条件紧密相关。试验证明饲料的营养因素对牛乳脂肪酸组成有显著的影响。

圆形贮奶罐 / 奶牛的牧场饲养 / 反式共轭脂肪酸和*n*-3脂肪酸 / 草场 / 奶牛

Résumé – Variabilité des acides gras de laits de collecte en fonction des pratiques alimentaires et des conduites d'élevage dans une zone française de semi-montagne. L'objectif de cette étude était, à l'échelle de laits de tournées, d'établir des relations entre les pratiques d'alimentation et les conduites d'élevage de différents systèmes de production et la composition en acides gras (AG) de ces laits. Les laits de 10 tournées dans le département français de la Haute-Loire (10 à 36 troupeaux par tournée) ont été prélevés, respectivement, deux et trois fois pendant l'hiver et la période de pâturage. Neuf variants des conditions de production des laits ont été définis : quatre pour la période hivernale (W1 à W4) et cinq pour la période de pâturage (G1 à G5). Au cours de l'année, l'acide ruménique était positivement corrélé à l'acide vaccénique (r = 0.99), à l'ensemble des autres isomères *trans* et *c*11 du C18:1, aux acides oléique (r = 0.79), linolénique (r = 0.82) et EPA. La matière grasse laitière provenant du pâturage avait une proportion plus élevée en AG *trans* totaux (incluant les C18:1 *trans*, les C18:2 non conjugués et le CLA-*c*9*t*11) et en C18:1 *cis* totaux, et une proportion plus faible en AG saturés à chaîne moyenne (−9.50 g·100 g^{-1} pour le C16:0 entre G5 et W1) et d'AG monoinsaturés (principalement C16:1-*c*9) que celle produite à partir des rations à base d'ensilage d'herbe (et supplémentées en concentré). De plus, l'*antéiso*-15, le C18:0, le C18:1-*c*9, les isomères *t*6+7+8, *t*9, *t*11 et *t*13+14 du C18:1, le CLA-*c*9*t*11 (r = 0.65), le C18:2-*t*11*c*15, le C18:3*n*-3 (r = 0.68) et l'EPA (r = 0.64) étaient positivement liés aux fourrages de prairie permanente (verts ou conservés) à l'échelle de l'année. Durant l'hiver, les isomères *trans* (*t*6+7+8, *t*10, *t*12 et *t*13+14) ou *cis* (*c*12, *c*13 et *t*16+*c*14) du C18:1 étaient positivement corrélés à la proportion d'ensilage de maïs dans la ration (r = 0.47 à 0.91). La grande variabilité de la composition en AG des laits des tournées dans cette étude était fortement liée aux variants d'alimentation et aux conditions d'élevage. Nos données confirment l'effet important des facteurs nutritionnels sur la composition en AG des laits de grand mélange, démontré au cours d'études expérimentales.

lait de collecte / alimentation et conduite d'élevage / acides gras trans, conjugués et *n*-3 / pâturage / vache laitière

1. INTRODUCTION

The nutritional quality of dairy products depends in part on their fatty acid (FA) composition. The properties of conjugated linoleic acids (CLA), whose main isomer, rumenic acid (*c*9*t*11-CLA), exhibits interesting features demonstrated in animal models, have been associated with the prevention of certain forms of cancer [44]. New findings have also been reported on the putative beneficial effects on cardiovascular diseases of increasing *n*-3 polyunsaturated fatty acid (PUFA) intake [36]. Moreover, branched-chain FA, such as *iso*-15, *anteiso*-15 and *iso*-16, have been shown to present anti-cancer activity in human breast cancer cell models [43]. However, dairy fat contains a large proportion of saturated FA, which if consumed in excess may play a role in the development of cardiovascular diseases [45]. The bad reputation of saturated FA must, however, be balanced by the fact that stearic acid has

little or no atherogenic effects [45]. Also, the alleged atherogenic effect of certain *trans* monounsaturated FA (mainly those occurring in partially hydrogenated vegetable oils) has not been confirmed for the main isomer present in milk, vaccenic acid (*t*11-C18:1) [35]. A recent report [1] recommends reducing *trans* FA intake, and labelling of *trans* FA has been advocated or is already mandatory in certain countries (e.g. Denmark and the United States). It is thus important for the dairy industry to characterise tanker milks in terms of saturated and *trans* FA composition.

Among the factors liable to modify bovine milk FA composition, intrinsic (animal breed, lactation and pregnancy stages [20, 26, 38]) and extrinsic (environmental, including feeding [6]) factors have minor and major influences, respectively. In contrast, cheese-making technology has a limited effect [21, 34]. Research carried out in experimental conditions has made it possible to quantify the specific effects of the nature and method of preserving forages, percentage of concentrate in the diet and lipid supplementation on the FA composition of individual milks [4, 6, 8]. Milks from pasture are richer in oleic acid, CLA and C18:3*n*-3 than those from concentrate or maize silage diets [7, 20]. However, the influence of the pasture seems to vary according to the growth stage and the botanical composition of the grass [12]. These observations made in experimental conditions have been confirmed at the level of commercial herds [33]. Nevertheless, there are very few reports on the year-round evolution of tanker milk mixtures collected from several herds. These round tanker milks are important for the dairy industry, accounting for 94% of the milk produced in France [10].

The objectives of this study were therefore to work on the round tanker scale (i) to characterise the average content and variability in the FA composition in milk fat from a geographical area (Haute-Loire, France) where a large range of milk production conditions occur together; and (ii) to correlate farm feeding practices of different management systems with milk FA composition.

2. MATERIALS AND METHODS

2.1. Characteristics of collection rounds

The study was conducted in 10 areas of the Haute-Loire department (Massif Central, France) that differ mainly by their forage system (grassland or maize silage) and altitude (440 to 1150 m). Within each of these 10 areas, a round (10 to 36 farms per round) for the collection of milks was set up so that samples were most representative of the diversity of milk production conditions in that area. Milk from the 10 collection round tankers was sampled five times in the course of the year 2002 at key times in animal feeding patterns: twice in the over-wintering period with diets based on preserved forage (February and March), and three times during the grazing period: turning-out to pasture with abundant young grass in May, summer drought in July and regrowths at the end of September.

2.2. Characterisation of milk production conditions in the collection rounds

The detailed characteristics of the collection rounds have been previously described [2]. Briefly, in order to characterise the milk production conditions on the day the milk was picked up, four surveys (a main one in winter and three additional ones in the grazing period) were carried out with each of the 204 farmers involved in the experiment. Questions were asked about (i) farm characteristics (area,

altitude, stable and milking equipment, milk quota, and quantity of milk delivered by each farm); (ii) herd characteristics (dairy cow numbers, calving distribution and breed); (iii) forage management (forage characteristics, forage harvesting and conservation, cutting and grazing periods, and the cropping pattern during 2001); and (iv) feeding the herd (types of feed, including concentrates).

To describe the production conditions associated with each sample originating from 10 to 36 herds differing in size and production level, we weighted the characteristics of the herds whose milk was collected by the tank truck by the contribution of the production of each herd to the milk tank (0.1 to 27.6%). We described diet on the basis of proportions of forages in the fodder ration calculated from the declared quantities the farmers dispensed or the estimated intake of standing grass at grazing (as described by Agabriel et al. [2]). The data concerning the production conditions of each milk sample ($n = 50, 10$ rounds on 5 collection dates) enabled us to describe the nature of the forage (proportion of permanent or temporary grassland, whole plant maize), the conservation method (grazed grass, hay, wrapped grass, grass silage, maize silage), the quantity of concentrate dispensed, the breed (Montbéliarde, Prim'Holstein) and the physiological stage of the animals. Thus, nine variants of milk production conditions (farm feeding and husbandry variants) were defined: four for the winter feeding period (W1 to W4) and five for the grazing period (G1 to G5) (Tab. I). Within each period, the variants were ranked in order of increasing proportion of permanent grassland forages (green or conserved) and decreasing proportion of whole plant maize in the diet. During the winter period, the proportions of hay and wrapped grass increased from W1 to W4 at the expense of maize silage and/or grass silage, whereas during the grazing period, the proportion of permanent grassland forages increased from G1 to G5 at the expense of either maize silage (G1) or temporary grassland (G2–G4). These feeding variants were linked in part to the altitude at which the milk was produced. They also corresponded to a shift from the feeding and husbandry systems of the plain, based on the use of maize silage (variants W1 and G1), with a slightly larger proportion of milk produced by Prim'Holstein animals and with the calving period in autumn, to mountain systems based on grass (mainly from permanent grassland) and associated with an increasing proportion of milk produced by Montbéliarde cows (W4 and G5) with calvings evenly distributed over the year.

2.3. Sampling and analyses

For each of the 50 collections (10 rounds × 5 dates), the milk from four or six milkings, stored in the exploitation tanks, was collected and pooled in tankers containing from 4082 to 32 998 L. One litre of milk was sampled from each tanker, stored at +4 °C without preserving agent and taken to the laboratory for analysis. One sub-sample (50 mL) was preserved in tubes with bronopol-B2 (Trillaud, Surgères, France) and stored at 4 °C for analysis. Fat and protein content (infrared spectrophotometry, Milkoscan 4000, Foss Electric, Hillerod, Denmark) were assayed in the fresh milk (CILAL, Theix, France) according to standard procedures [3].

Another sub-sample (3 mL) was stored at −20 °C until it was lyophilised (Thermovac TM-20, Froilabo, Ozoir-La-Ferrière, France) for analysis of FA composition. Fatty acids in lyophilised milk were directly methylated according to Loor et al. [29]. Samples were injected by auto-sampler into a Trace-GC 2000 series gas chromatograph equipped with

Table I. Characteristics of milk production conditions according to nine feeding and husbandry variants.

Feeding and husbandry variants	Winter feeding period				Grazing period					rSD[1]	Stat. sign.[2]	Grazing –Winter	rSD[1]	Stat. sign.[2]
Number of samples	W1 $n=4$	W2 $n=4$	W3 $n=8$	W4 $n=4$	G1 $n=7$	G2 $n=6$	G3 $n=6$	G4 $n=5$	G5 $n=6$					
Nature of forages (% of forage DM)														
Permanent grassland	19^{a}	21^{a}	40^{b}	97^{e}	34^{b}	54^{c}	60^{c}	76^{d}	96^{e}	7	***	20	26	*
Temporary grassland	19^{b}	43^{d}	58^{e}	3^{a}	32^{cd}	39^{d}	40^{d}	23^{bc}	3^{a}	9	***	−8	19	ns
Maize silage	62^{c}	35^{b}	2^{a}	0^{a}	33^{b}	7^{a}	0^{a}	0^{a}	1^{a}	4	***	−11	19	+
Mode of conservation of forages (% of forage DM)														
Grazing	0^{a}	0^{a}	0^{a}	0^{a}	49^{b}	71^{c}	73^{c}	68^{c}	77^{c}	8	***			
Grass hay	16^{ab}	20^{ab}	38^{c}	65^{d}	11^{a}	15^{ab}	21^{ab}	27^{b}	21^{ab}	8	***	−17	14	***
Wrapped grass	2^{a}	4^{a}	27^{b}	35^{c}	1^{a}	2^{a}	5^{a}	4^{a}	1^{a}	6	***	−16	10	***
Grass silage	20^{b}	41^{d}	33^{c}	0^{a}	6^{a}	4^{a}	3^{a}	1^{a}	0^{a}	6	***	−22	11	***
Maize silage	62^{c}	35^{b}	2^{a}	0^{a}	33^{b}	7^{a}	0^{a}	0^{a}	1^{a}	4	***	−11	19	+
Concentrate ($kg \cdot cow^{-1} \cdot d^{-1}$)	4.2^{bc}	4.5^{cd}	5.0^{d}	3.3^{ab}	3.7^{b}	3.3^{ab}	3.4^{ab}	3.4^{ab}	2.6^{a}	0.5	***	−1.1	0.7	***
Altitude[3] (m)	454	782	1024	1129	474	840	915	1019	1122			−26	260	ns
Breed of dairy cows (%)														
Montbéliarde	37^{a}	53^{b}	80^{cd}	97^{d}	55^{b}	61^{bc}	76^{c}	80^{cd}	97^{d}	12	***		21	
Prim'Holstein	61^{d}	43^{c}	18^{ab}	2^{a}	45^{c}	36^{c}	21^{b}	17^{ab}	2^{a}	11	***		21	
Physiological stage (months)	4.8^{a}	5.2^{abc}	5.1^{ab}	5.9^{cd}	6.2^{d}	5.9^{cd}	5.6^{bcd}	5.6^{bcd}	5.2^{abc}	0.3	***	0.5	0.5	***

[1] rSD = residual standard deviation.
[2] Statistical significance: ns: $P > 0.10$; +: $P \leqslant 0.10$; *: $P \leqslant 0.05$; **: $P \leqslant 0.01$; ***: $P \leqslant 0.001$.
[3] Altitude of localisation of average herds.
[a,b,c,d] Means with different letters are different at $P < 0.05$ (Student-Newman-Keuls t-test).

a flame ionisation detector (Thermo Finnigan, Les Ulis, France). Methyl esters from all the samples were separated on a 100 m × 0.25 mm i.d. fused-silica capillary column (CP-Sil 88, Chrompack, Middelburg, The Netherlands). The injector temperature was maintained at 250 °C and the detector temperature at 255 °C. The initial oven temperature was held at 70 °C for 1 min, increased by 5 °C·min^{-1} to 100 °C (held for 2 min), and then increased by 10 °C·min^{-1} to 175 °C (held for 40 min), and 5 °C·min^{-1} to a final temperature of 225 °C (held for 15 min). The carrier gas was hydrogen. Identification of *trans*-C18:1, non-conjugated C18:2 and CLA isomers was as described in [29]. A reference standard butter (CRM 164, Commission of the European Communities, Community Bureau of Reference, Brussels, Belgium) was used to estimate correction factors for short-chain FA (C4:0 to C10:0).

2.4. Statistical analysis

To describe the relationships between the FA, data were analysed using principal component analysis (PCA - SPAD 6.0, 2005) with 49 FA having concentrations higher than 0.01% (% of total FA) used as active variables (C4:0, C6:0, C8:0, C10:0, *c*9-C10:1, C12:0, C13:0, *iso*-14, C14:0, *iso*-15, *anteiso*-15, *c*9-C14:1, C15:0, *iso*-16, C16:0, *t*9-C16:1, *c*9-C16:1, *iso*-17, *anteiso*-17, C17:0, *iso*-18, *c*9-C17:1, C18:0, *trans* (*t*6+7+8, *t*9, *t*10, *t*11, *t*12 and *t*13+14) and *cis* isomers of C18:1 (*c*9, *c*11, *c*12, *c*13 and *c*15) and *t*16+*c*14-C18:1, *c*9*t*13-C18:2, *c*9*t*12-C18:2, *t*11*c*15-C18:2, *c*9*c*12-C18:2, C20:0, C18:3*n*-3, *c*9*c*11-CLA, *c*9*t*11-CLA, C20:3*n*-6, C20:4*n*-6, C22:2*n*-6, C20:5*n*-3, C22:0, and C22:5*n*-3) and with eight milk production conditions projected on the principal components (proportion of grazed grass, hay, grass silage, wrapped grass, maize silage, permanent grassland forages, quantity of concentrate and average physiological stage of the average herd). Two significant principal components (PC) were extracted describing 44% (PC1) and 20% (PC2) of the total variation in milk FA percentages and milk production conditions. The variables best correlated with these two PC were selected. Thus, 37 FA (of the 49 FA) and seven milk production conditions are represented. Relationships between FA and the milk production conditions were also studied using correlation calculations (Proc corr [41]). When correlation coefficients were high ($r > 0.65$), some interesting relationships between FA and milk production conditions are represented by regression equations.

To test the differences between winter and grazing periods, data were processed by variance analysis using the GLM procedure [41]. Differences between farm feeding and husbandry variants were determined by variance analysis using the GLM procedure [41] and using the pairwise Student-Newman-Keuls's *t*-test when the probability was < 0.05.

3. RESULTS

3.1. Variability in milk fatty acid composition and correlations between milk fatty acid percentages

The milks were composed of an average of 65% even saturated FA (sum of C4:0 to C22:0), 5% linear odd medium-chain FA and branched-chain FA (BCFA), 25% monounsaturated FA (including 3% of *trans* C18:1) and 4% PUFA. This composition indicated an atherogenic saturated FA index (ASFAI = C12:0 + 4 × C14:0 + C16:0) equal to 79% (Tab. II), with a variation coefficient of 8%. The variability differed widely according to the FA family. It was low for the short- and medium-chain FA (< 8% and 7–12%, for C4:0 to C8:0 and

Table II. Chemical and fatty acid composition of milks from the 50 round tankers.

	Average	Standard deviation	Minimum	Maximum	Variation coefficient (%)
Chemical characteristics					
Protein content ($g \cdot kg^{-1}$)	32.38	0.85	30.70	34.40	2.61
Fat content ($g \cdot kg^{-1}$)	42.55	4.36	33.50	53.60	10.24
Fatty acids ($g \cdot 100\ g^{-1}$ of total FA)					
C4:0	3.18	0.25	2.73	3.88	7.86
C6:0	2.24	0.12	2.04	2.63	5.44
C8:0	1.32	0.08	1.16	1.56	6.29
C10:0	3.02	0.24	2.50	3.54	7.98
*c*9-C10:1	0.29	0.02	0.24	0.34	7.06
C12:0	3.35	0.29	2.78	3.82	8.79
C13:0	0.19	0.02	0.15	0.22	10.94
iso-14	0.15	0.03	0.09	0.23	20.36
C14:0	11.42	0.71	10.23	12.57	6.18
iso-15	0.34	0.05	0.20	0.46	16.31
anteiso-15	0.60	0.08	0.41	0.75	13.28
*c*9-C14:1	0.91	0.10	0.69	1.08	10.74
C15:0	1.30	0.09	1.14	1.49	7.17
iso-16	0.33	0.05	0.24	0.48	14.57
C16:0	30.22	3.67	24.41	36.41	12.13
iso-17	0.44	0.06	0.30	0.51	12.72
*t*9-C16:1	0.11	0.05	0.05	0.25	46.66
anteiso-17	0.65	0.07	0.43	0.77	10.35
*c*9-C16:1	1.39	0.17	1.04	1.68	12.00
C17:0	0.66	0.05	0.52	0.77	8.30
iso-18	0.05	0.01	0.04	0.07	15.49
*c*9-C17:1	0.27	0.02	0.22	0.32	8.38
C18:0	9.65	1.04	8.10	11.45	10.73
trans isomers of C18:1					
*t*6+7+8	0.15	0.04	0.05	0.22	26.83
*t*9	0.19	0.04	0.12	0.28	20.99
*t*10	0.21	0.06	0.10	0.33	28.48
*t*11	1.81	0.98	0.65	4.09	54.00
*t*12	0.19	0.05	0.11	0.28	25.85
*t*13+14	0.47	0.11	0.20	0.66	24.22
cis isomers of C18:1					
*c*9	18.67	1.70	15.49	21.26	9.12
*c*11	0.51	0.06	0.42	0.63	11.40
*c*12	0.11	0.03	0.06	0.23	28.88
*c*13	0.05	0.01	0.02	0.07	24.31
*t*16+*c*14	0.26	0.06	0.12	0.36	23.40
*c*15	0.08	0.03	0.00	0.14	41.76

Table II. Continued.

	Average	Standard deviation	Minimum	Maximum	Variation coefficient (%)
*c*9*t*13-C18:2	0.14	0.05	0.06	0.24	34.82
*c*9*t*12-C18:2	0.03	0.04	0.00	0.09	120.97
*t*11*c*15-C18:2	0.19	0.14	0.00	0.54	73.70
*c*9*c*12-C18:2	1.36	0.08	1.15	1.53	5.89
C20:0	0.13	0.02	0.09	0.17	13.61
C18:3*n*-3	0.67	0.18	0.22	0.95	26.75
*c*9*t*11−CLA	0.83	0.43	0.29	1.98	51.36
*c*9*c*11-CLA	0.03	0.03	0.00	0.11	102.98
tt-CLA	0.01	0.01	0.00	0.03	140.37
C20:2*n*-6	0.00	0.01	0.00	0.02	174.78
C22:0	0.07	0.01	0.05	0.09	15.35
C20:3*n*-6	0.06	0.01	0.04	0.08	13.20
C20:3*n*-3	0.01	0.01	0.00	0.02	101.75
C20:4*n*-6	0.09	0.01	0.07	0.12	14.67
C22:2*n*-6	0.03	0.01	0.00	0.06	32.12
C20:5*n*-3	0.07	0.01	0.04	0.10	18.84
C22:4*n*-6	0.01	0.02	0.00	0.08	154.27
C22:5*n*-3	0.09	0.01	0.05	0.11	15.97
C22:6*n*-3	0.00	0.01	0.00	0.10	454.71
Saturated FA	64.61	3.73	59.08	71.39	5.77
Linear odd short-chain FA[1]	0.13	0.03	0.08	0.19	22.75
Linear odd medium-chain FA + BCFA[2]	4.70	0.39	3.67	5.59	8.29
Total *cis* C18:1[3]	19.42	1.77	16.24	22.18	9.11
Total *trans* C18:1[4]	3.01	1.20	1.42	5.69	39.87
Total *trans* FA[3]	4.22	1.82	1.93	8.56	43.13
ASFAI[5]	79.25	6.44	69.13	87.64	8.13

[1] Linear odd short-chain FA = C5:0 + C7:0 + C9:0 + C11:0.
[2] Linear odd medium-chain FA and branched-chain FA (BCFA) = C13:0 + C15:0 + C17:0 + *iso*-14 + *iso*-15 + *anteiso*-15 + *iso*-16 + *iso*-17 + *anteiso*-17 + *iso*-18.
[3] Except for *t*16+*c*14-C18:1.
[4] Total *trans* FA = sum of *trans* C18:1 + *trans* non-conjugated C18:2 + *tt*-CLA + *c*9*t*11-CLA.
[5] ASFAI = atherogenic saturated fatty acid index = C12:0 + 4 × C14:0 + C16:0 from Chilliard et al. [9].

C10:0 to C14:0, respectively), as for C16:0 and C18:0. The odd- and branched-chain FA did not vary widely (8%). Oleic acid presented a variation coefficient of 9%. The PUFA were either moderately variable (linoleic acid, 6%) or highly variable (linolenic and rumenic acids, 27% and 51%, respectively). The variability in the *trans* isomers of C18:1 was comparable and relatively high (21% to 29%), except for *t*11-C18:1 with the highest variation coefficient (54%). The *c*11 to *c*13 isomers of C18:1 were moderately variable (11% to 29%). The variation coefficients of saturated long-chain FA (C20:0 and C22:0) were 14% to 15%, and those of EPA and

docosapentaenoic acid (C22:5*n*-3, DPA) were 19% and 16%, respectively. The 20- and 22-carbon FA were moderately to highly variable; 13% to 455% for docosahexaenoic acid (C22:6*n*-3, DHA), probably because of their very low concentrations (0.09 to 0.01 g·100 g^{-1}).

The PCA performed on the different FA percentages in the milk fat gives an overview of the main correlations observed between the FA. Two significant principal components (PC) could be extracted describing 44% (PC1) and 20% (PC2) of the total variation in milk FA composition (Fig. 1A). PC1 differentiates milks richer in saturated (C10:0, C12:0, C13:0, C14:0 and C16:0) and monounsaturated FA (*c*9-C10:1, *c*9-C14:1 and *c*9-C16:1) medium-chain FA from milks richer in stearic, oleic, vaccenic, rumenic and linolenic acids, *c*9*c*11-CLA, *t*11*c*15-C18:2, *c*9*t*13-C18:2, *t*9-C16:1, and *trans* (*t*6+7+8, *t*9, t10, *t*12, *t*13+14 and *t*16+*c*14) and *cis* (*c*11 and *c*15) isomers of C18:1. The milks of the first cluster are closely associated with grass silage and concentrate, whereas those of the second cluster are linked to grazed grass. PC2 differentiates milks richer in linear odd medium-chain FA (C15:0 and C17:0), odd or even branched-chain FA (*iso*-14, *iso*-15, *anteiso*-15, *iso*-16, *anteiso*-17 and *iso*-18) and EPA, from milks richer in *t*10, *t*12, *t*13, *t*16 + *c*14 and *c*12 isomers of C18:1. The milks of the third cluster are closely associated with hay, wrapped grass and permanent grassland forages, and those of the fourth cluster are linked to maize silage. Figure 1B shows the biplot resulting from PCA applied to the different milk samples. PC1 differentiates milks from grazing (G2 to G5) from those from the winter period. Milks from G1 (with 49% forage coming from grazing) lay between milks from winter and grazing periods. PC2 mainly differentiates W1 milks from W4 milks, i.e. milks produced in winter with diets rich in maize silage, as opposed to diets rich in hay.

Among the different correlations, on the scale of the year, we observed positive links between rumenic acid and vaccenic acid (Fig. 2A), all the other *trans* isomers of C18:1 (r = 0.58 to 0.89, with the highest value for *t*9 isomer), *c*11 isomer (r = 0.62), oleic acid (r = 0.79), linolenic acid (r = 0.82, Fig. 2B), and EPA (r = 0.56). However, these general correlations varied according to the period: during the winter feeding period, rumenic acid was less closely correlated with linolenic acid (r = 0.66 vs. 0.84, $P < 0.01$), and more strongly to *c*9-C18:1 (r = 0.86, $P < 0.001$ vs. 0.31, $P < 0.10$) and EPA (r = 0.70 vs. 0.64, $P < 0.001$) than during the grazing period. Linolenic acid was strongly correlated with EPA over the year (Fig. 2C). This correlation was, however, slightly higher during the winter (r = 0.94, $P < 0.001$) than during the grazing period (r = 0.82, $P < 0.001$).

3.2. Effects of farm feeding and husbandry variants on milk fatty acid composition

The percentages of short-chain FA (C4:0 to C8:0) varied slightly according to the nine feeding and husbandry variants (+0.4 g·100 g^{-1} between W1 and G5 for C4:0, P = 0.01, Tab. III). The percentages of the C10:0 to C14:0 FA were significantly higher during winter feeding than during the grazing period and they decreased linearly from W1 to G5. The percentage of C16:0 decreased strongly from W1 to G5 (−9.50 g·100 g^{-1}), with a significant effect of grazing vs. winter feeding period (−6.4 g·100 g^{-1}, $P < 0.001$). The sum of C5:0 to C11:0 decreased from W1 to W4, and slightly from G1 to G5. In contrast, the sum of linear odd medium-chain FA and BCFA gradually increased from W1 to W4, and from G1 to G5, with no grazing vs. winter feeding effect.

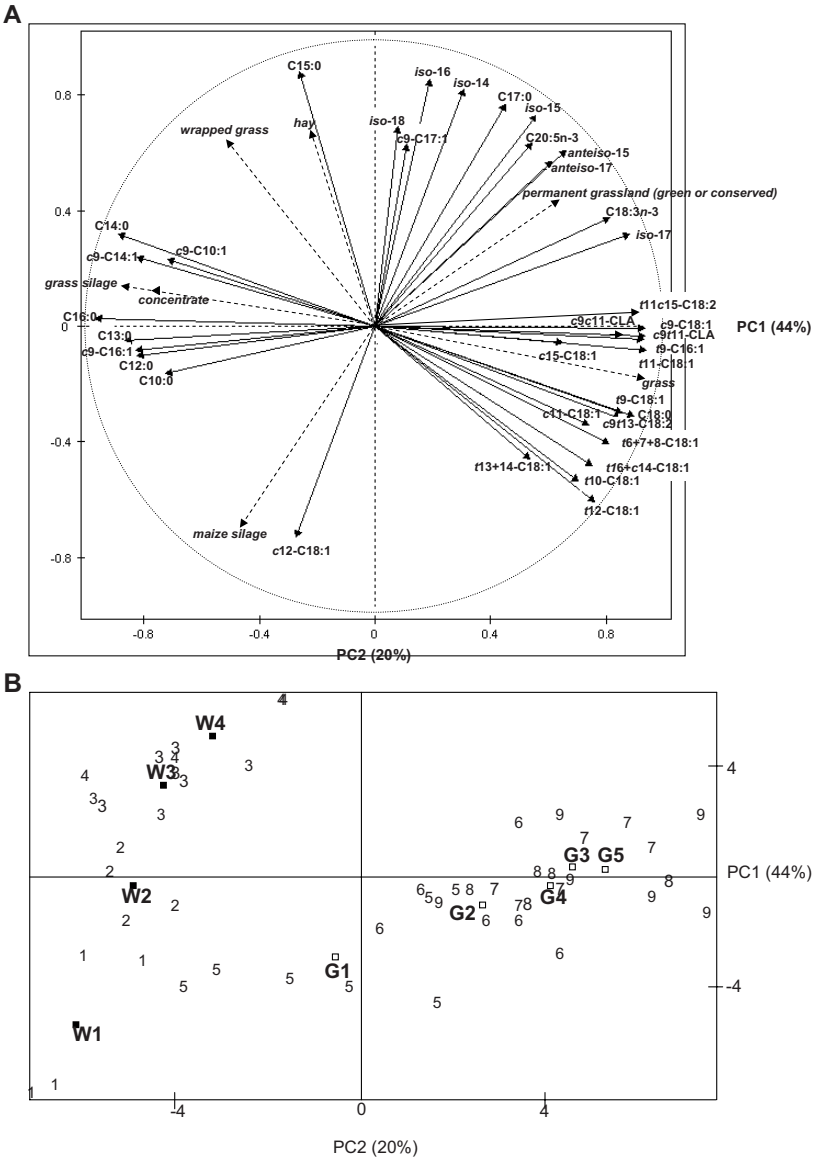

Figure 1. A: Representation of the relationships among milk percentages of 49 fatty acids and feeding management practices (proportion of grazed grass, hay, grass silage, wrapped grass, maize silage, permanent grassland forages (green or conserved), or kg·d^{-1} of concentrate) derived from a principal component analysis. Milk fatty acids (% of total fatty acids) (normal characters), feeding management practices (italic characters). Plot of variables is projected on the first two principal components (PC). B: Plots on the PC1and PC2 showing the distribution of milk samples from the nine farm feeding and husbandry variants. Each number represents the individual milk samples and each square represents the barycentre of each variant. The W1 variant is represented by the number 1, W2 by 2, W3 by 3, W4 by 4, G1 by 5, G2 by 6, G3 by 7, G4 by 8 and G5 by 9.

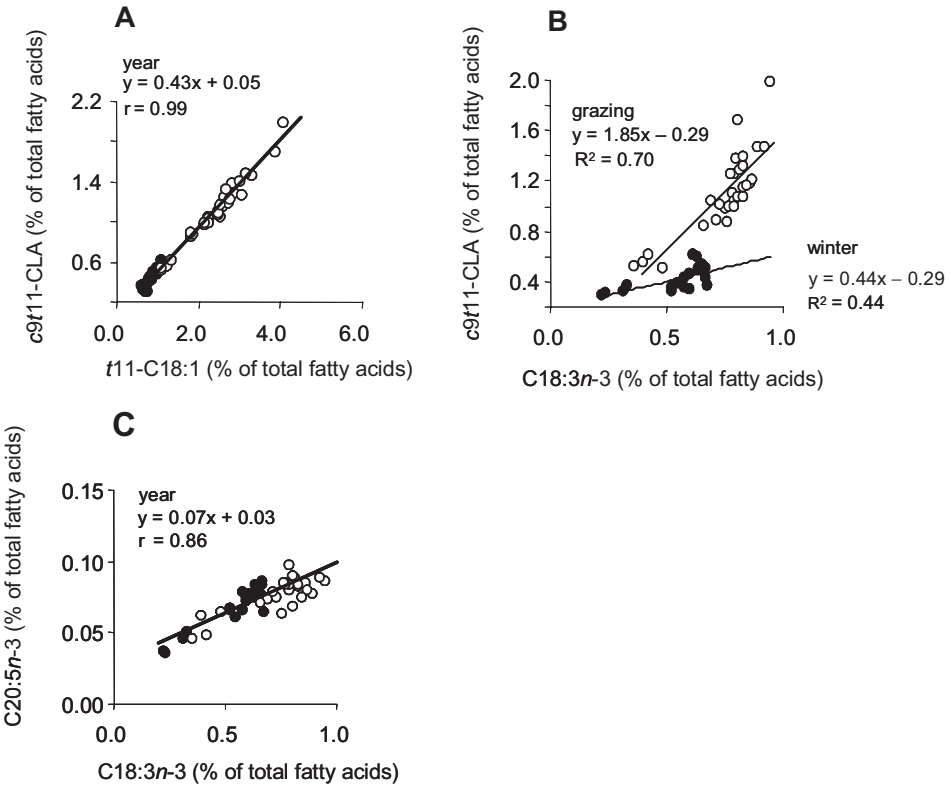

Figure 2. Relationships between *t*11-C18:1, *c*9*t*11-CLA, C18:3*n*-3 and C20:5*n*-3 percentages in cow's milks from the round tankers. • Winter feeding period, ○ grazing period.

The grazing vs. winter feeding milks were richer in stearic acid (+1.9 g·100 g^{-1}, $P < 0.001$). Within the period this percentage decreased non-significantly from W1 to W4 and increased significantly from G1 to G5. The percentage of oleic acid increased from W1 to G5 (+3.30 g·100 g^{-1}, $P < 0.001$), with an average difference between grazing vs. winter feeding milks of 2.9 g·100 g^{-1} ($P < 0.001$). All the *trans* isomers of C18:1 were significantly higher in grazing than in winter feeding milks. Their percentages decreased slightly from W1 to W4 (–0.03 to –0.08 g·100 g^{-1}), except for *t*11, and they increased from G1 to G5 (+0.02 to +0.09 g·100 g^{-1}). The *t*11-C18:1 increased strongly from W1 to G5 (+2.59 g·100 g^{-1}). The *cis* isomers (except for *c*12) were also higher in grazing than in winter feeding milks. The *c*12 and *c*13 isomers decreased slightly from W1 to W4 and from G1 to G5, the *c*11 isomer decreasing only from W1 to W4. Percentages of *c*9*t*13-, *t*11*c*15-C18:2 and linolenic acid increased notably from G1 to G5 (+0.09 to +0.20 g·100 g^{-1}), with a significant grazing vs. winter feeding period effect. The *c*9*t*11-CLA varied markedly according to the feeding and husbandry variants (+1.2 g·100 g^{-1} from W1 to G5). The *c*9*c*11 and *tt* isomers of CLA were only detectable during the grazing period (0.02 to 0.07 g·100 g^{-1}). The percentages of C20:3*n*−6 and C20:4*n*−6 decreased very

slightly from W1 to G5. The main variation in EPA was a decrease with maize silage (W1, W2 and G1) compared with other groups (0.05 vs. 0.08 g·100 g^{-1}, $P < 0.05$, and Fig. 1).

A more direct comparison between winter vs. grazing periods could be made by comparing W2 and G1, i.e. the effect of grass silage vs. grazing grass, the other milk production conditions being closely similar, although the lactation stage differed by 1 month (5.2 vs. 6.2 months). This comparison supports data from PCA: grazing decreased C14:0 and C16:0, and increased *iso*-17, *t*9-C16:1, total *cis* C18:1 and total *trans* FA (including total *trans* C18:1, *c*9*t*13-, *t*11*c*15-C18:2 and *c*9*t*11-CLA). Another comparison can be made between G5 vs. W4 variants, comprising the same collection rounds. Milks from G5 (grazing) compared with those from W4 (with predominance of hay) were poorer in *c*9-C10:1 and *c*9-C14:1, C13, *iso*-14, C15:0, *iso*-16, *c*9-C16:1, *iso*-18, *c*9-C17:1 and saturated FA (especially C14:0 and C16:0) and were richer in total *trans* FA (especially total *trans* C18:1, *c*9*t*13- and *t*11*c*15-C18:2 and *c*9*t*11-CLA), total *cis* C18:1 and C18:3*n*-3.

3.3. Correlations between milk fatty acid percentages and the main forages consumed by the cows

The saturated FA (C10:0 to C16:0, Figs. 1 and 3A) were positively correlated with the proportion of grass silage (r = 0.42 to 0.76), and the amount of concentrate (r = 0.45 to 0.67) in the diet. However, during the grazing period, only C16:0 (of the saturated FA) was positively correlated with grass silage ($r = 0.69, P < 0.001$) and the amount of concentrate (r = 0.46, $P < 0.01$). The *c*9-C10:1, *c*9-C14:1, C14:0, C15:0 and C16:0 were positively correlated with the proportion of wrapped grass (r = 0.45 to 0.70, and Fig. 1) over the year. Most of the linear odd medium-chain FA and BCFA (except for C13:0, *iso*-17 and *anteiso*-17) were correlated with the proportion of hay in the diet (r = 0.38 to 0.71, and Fig. 3B for *iso*-16 and hay) over the year. In addition, this FA family was positively correlated with the proportion of permanent grassland forages in the diet (r = 0.38 to 0.84, except for C13:0 and C15:0, and Fig. 4A for *anteiso*-15). On the scale of the year, the stearic and oleic acids were moderately correlated with permanent grassland forages (r = 0.39 and 0.49, respectively). The correlations differed, however, according to the period: the C18:0 percentage was correlated with permanent grassland forages, negatively during winter and positively during the grazing period ($-0.50, P < 0.05$ and $+0.52$, $P < 0.01$, respectively, Fig. 4B). The relation between oleic acid and permanent grassland forages appeared only during the winter feeding period ($r = 0.64, P < 0.01$). The *c*9*t*11-CLA (r = 0.65), C18:3*n*-3 (r = 0.68), *t*11*c*15-C18:2 (r = 0.65) and EPA (r = 0.64) were positively correlated with permanent grassland forages during both winter feeding and grazing periods (Figs. 4C, 4D, 4E and 4F, respectively).

Concerning the *trans* isomers of C18:1, all FA, except for *t*10 and *t*12, were positively correlated with permanent grassland forages (r = 0.32 to 0.65, Fig. 4G for *t*11 and permanent grassland forages) over the year. Also, the *trans* isomers were negatively correlated with hay (r = −0.59 to −0.31, except for *t*11), grass silage (r = −0.76 to −0.54), temporary grassland forages (r = −0.47 to −0.30), amount of concentrate (r = −0.71 to −0.42) in the diet, and positively with grazed grass (r = 0.66 to 0.89).

During the grazing period, *trans* isomers were correlated with grazed grass (r = 0.37 to 0.63, except for *t*10 and *t*12) and permanent grassland forages (r = 0.62 to 0.83, except for *t*10 and *t*13+14). During winter, the *t*12 isomer was negatively

Table III. Fat content and fatty acid composition of milks from the round tankers according to nine feeding and husbandry variants and season.

	Winter feeding period				Grazing period									
Feeding and husbandry variants	W1	W2	W3	W4	G1	G2	G3	G4	G5	rSD[1]	Sign.[2]	Grazing-winter	rSD	Sign.[3]
Number of samples	$n = 4$	$n = 4$	$n = 8$	$n = 4$	$n = 7$	$n = 6$	$n = 6$	$n = 5$	$n = 6$					
Protein content ($g \cdot kg^{-1}$)	32.5	32.0	32.2	31.6	32.9	32.7	31.8	32.9	32.5	0.8	+	0.5		+
Fat content ($g \cdot kg^{-1}$)	46.2	45.8	39.7	46.9	43.4	40.9	42.6	41.2	40.0	4.0	ns	−2.5		+
Fatty acids (% of total FA)														
C4:0	3.11^{a}	3.06^{a}	3.04^{a}	3.35^{ab}	3.12^{a}	3.19^{ab}	3.19^{ab}	3.8^{a}	3.54^{b}	0.21	**	0.11	0.25	ns
C6:0	2.3	2.3	2.2	2.3	2.2	2.2	2.1	2.2	2.3	0.12	ns	−0.05	0.12	ns
C8:0	1.4^{b}	1.4^{ab}	1.3^{ab}	1.4^{ab}	1.3^{ab}	1.3^{ab}	1.2^{a}	1.3^{ab}	1.3^{ab}	0.08	+	−0.05	0.08	*
C10:0	3.3^{c}	3.2^{bc}	3.1^{bc}	3.1^{bc}	3.0^{abc}	3.0^{abc}	2.7^{a}	3.0^{abc}	2.9^{ab}	0.20	***	−0.27	0.204	***
*c*9-C10:1	0.29^{ab}	0.29^{abc}	0.30^{bc}	0.31^{c}	0.29^{ab}	0.28^{ab}	0.26^{a}	0.27^{ab}	0.27^{ab}	0.02	***	−0.02	0.017	***
C12:0	3.8^{d}	3.6^{cd}	3.6^{cd}	3.5^{bcd}	3.3^{bc}	3.3^{abc}	3.0^{a}	3.3^{abc}	3.1^{ab}	0.2	***	−0.40	0.22	***
C13:0	0.21^{d}	0.20^{cd}	0.20^{cd}	0.19^{bc}	0.19^{bc}	0.18^{ab}	0.16^{a}	0.18^{ab}	0.16^{a}	0.01	***	−0.03	0.014	***
iso-14	0.11^{a}	0.12^{ab}	0.16^{cd}	0.20^{e}	0.12^{a}	0.14^{bc}	0.16^{cd}	0.15^{bc}	0.18^{d}	0.02	***	0	0.031	ns
C14:0	11.9^{c}	12.0^{c}	12.3^{c}	12.3^{c}	11.3^{b}	11.0^{ab}	10.7^{a}	10.9^{ab}	10.8^{ab}	0.34	***	−1.21	0.38	***
iso-15	0.24^{a}	0.28^{b}	0.34^{cd}	0.40^{e}	0.30^{bc}	0.33^{cd}	0.36^{de}	0.34^{cd}	0.40^{e}	0.03	***	0.02	0.054	ns
anteiso-15	0.45^{a}	0.51^{b}	0.59^{cd}	0.68^{e}	0.55^{bc}	0.60^{cd}	0.63^{de}	0.65^{de}	0.70^{e}	0.04	***	0.06	0.074	**
*c*9-C14:1	0.93^{b}	0.95^{bc}	1.00^{cd}	1.04^{d}	0.95^{bc}	0.89^{b}	0.82^{a}	0.80^{a}	0.77^{a}	0.04	***	−0.13	0.072	***
C15:0	1.2^{a}	1.3^{b}	1.4^{c}	1.4^{c}	1.2^{ab}	1.2^{ab}	1.3^{b}	1.3^{b}	1.3^{b}	0.05	***	−0.10	0.08	***
iso-16	0.27^{a}	0.30^{ab}	0.36^{c}	0.41^{d}	0.28^{a}	0.32^{abc}	0.34^{bc}	0.33^{bc}	0.36^{c}	0.03	***	−0.02	0.048	ns
C16:0	35.3^{d}	34.7^{d}	34.0^{d}	32.4^{c}	30.4^{b}	27.6^{a}	27.3^{a}	26.6^{a}	25.8^{a}	1.3	***	−6.42	1.85	***
iso-17	0.33^{a}	0.36^{a}	0.40^{b}	0.45^{cd}	0.43^{bc}	0.47^{d}	0.48^{d}	0.49^{d}	0.48^{d}	0.03	***	0.08	0.04	***
*t*9-C16:1	0.05^{a}	0.06^{a}	0.06^{a}	0.08^{ab}	0.10^{b}	0.13^{c}	0.15^{c}	0.15^{c}	0.19^{d}	0.02	***	0.08	0.03	***
anteiso-17	0.49^{a}	0.61^{b}	0.66^{bc}	0.66^{bc}	0.64^{bc}	0.67^{bc}	0.71^{c}	0.69^{bc}	0.66^{bc}	0.04	***	0.06	0.06	**
*c*9-C16:1	1.6^{c}	1.5^{c}	1.5^{c}	1.5^{c}	1.5^{c}	1.3^{b}	1.3^{b}	1.2^{b}	1.1^{a}	0.08	***	−0.21	0.13	***
C17:0	0.57^{a}	0.62^{ab}	0.68^{c}	0.70^{c}	0.61^{ab}	0.65^{bc}	0.70^{c}	0.69^{c}	0.69^{c}	0.04	***	0.01	0.05	ns
iso-18	0.05^{a}	0.05^{a}	0.06^{ab}	0.07^{b}	0.05^{a}	0.05^{a}	0.06^{ab}	0.06^{ab}	0.05^{a}	0.007	***	0	0.008	*
*c*9-C17:1	0.24^{a}	0.25^{ab}	0.28^{bc}	0.29^{c}	0.26^{abc}	0.26^{abc}	0.28^{bc}	0.27^{abc}	0.25^{ab}	0.02	**	0	0.02	ns
C18:0	8.9^{a}	8.8^{a}	8.3^{a}	8.4^{a}	9.9^{b}	10.4^{bc}	10.6^{bc}	10.4^{bc}	10.7^{c}	0.42	***	1.86	0.48	***

Table III. Continued.

trans isomers of C18:1														
*t*6+7+8	0.14^{bc}	0.13^{b}	0.09^{a}	0.10^{a}	0.16^{cd}	0.17^{cd}	0.18^{de}	0.17^{cde}	0.20^{e}	0.02	***	0.06	0.025	***
*t*9	0.17^{b}	0.13^{a}	0.14^{ab}	0.15^{ab}	0.20^{c}	0.20^{c}	0.21^{c}	0.21^{c}	0.24^{d}	0.02	***	0.06	0.023	***
*t*10	0.21^{b}	0.15^{a}	0.14^{a}	0.14^{a}	0.24^{b}	0.23^{b}	0.23^{b}	0.25^{b}	0.26^{b}	0.04	***	0.09	0.04	***
*t*11	0.73^{a}	0.70^{a}	0.84^{a}	1.08^{a}	1.58^{b}	2.27^{c}	2.53^{c}	2.77^{c}	3.32^{d}	0.34	***	1.61	0.57	***
*t*12	0.19^{c}	0.16^{b}	0.12^{a}	0.12^{a}	0.22^{cd}	0.22^{d}	0.22^{cd}	0.24^{d}	0.24^{d}	0.02	***	0.09	0.03	***
*t*13+14	0.43^{abc}	0.34^{a}	0.37^{ab}	0.35^{a}	0.49^{bc}	0.53^{c}	0.50^{bc}	0.57^{c}	0.58^{c}	0.08	***	0.16	0.08	***
Total	1.87^{a}	1.61^{a}	1.70^{a}	1.94^{a}	2.89^{b}	3.61^{c}	3.86^{cd}	4.21^{d}	4.85^{e}	0.37	***	2.08	0.64	***
cis isomers of C18:1														
*c*9	16.3^{a}	16.6^{ab}	16.1^{ab}	17.7^{b}	19.1^{c}	19.8^{c}	20.5^{c}	20.2^{c}	19.6^{c}	0.8	***	2.89	0.93	***
*c*11	0.50^{b}	0.44^{a}	0.44^{a}	0.46^{a}	0.55b^{cd}	0.54^{bcd}	0.56^{cd}	0.58^{d}	0.51^{bc}	0.03	***	0.09	0.04	***
*c*12	0.18^{c}	0.13^{b}	0.09^{a}	0.09^{a}	0.13^{ab}	0.11^{ab}	0.10^{ab}	0.11^{ab}	0.10^{ab}	0.02	***	−0.01	0.03	ns
*c*13	0.06^{c}	0.04^{ab}	0.03^{a}	0.03^{a}	0.06^{c}	0.05^{c}	0.05^{bc}	0.05^{c}	0.04^{ab}	0.007	***	0.01	0.01	***
*t*16+*c*14	0.22^{b}	0.22^{b}	0.19^{ab}	0.17^{a}	0.29^{c}	0.32^{c}	0.30^{c}	0.31^{c}	0.31^{c}	0.03	***	0.11	0.03	***
*c*15	0.05^{a}	0.07^{ab}	0.06^{a}	0.05^{a}	0.09^{abc}	0.12^{c}	0.12^{bc}	0.09^{abc}	0.08^{abc}	0.03	***	0.04	0.03	***
Total[4]	17.07^{a}	17.23^{a}	17.70^{a}	18.34^{a}	19.95^{b}	20.63^{b}	21.33^{b}	21.07^{b}	20.36^{b}	0.88	***	3.02	0.95	***
*c*9*t*13-C18:2	0.09^{a}	0.10^{a}	0.09^{a}	0.08^{a}	0.16^{b}	0.19^{b}	0.18^{b}	0.19^{b}	0.19^{b}	0.02	***	0.09	0.02	***
*c*9*t*12-C18:2	0.01^{ab}	0.05^{ab}	0.0^{a}	0.0^{a}	0.03^{ab}	0.08^{b}	0.04^{ab}	0.03^{ab}	0.04^{ab}	0.03	**	0.03	0.03	**
*t*11*c*15-C18:2	0.0^{a}	0.0^{a}	0.10^{b}	0.11^{b}	0.14^{b}	0.29^{c}	0.31^{c}	0.29^{c}	0.38^{c}	0.06	***	0.22	0.09	***
*c*9*c*12-C18:2	1.44	1.30	1.32	1.30	1.33	1.36	1.41	1.43	1.37	0.07	*	0.04	0.08	+
C20:0	0.12	0.14	0.14	0.13	0.13	0.14	0.14	0.13	0.13	0.02	ns	0	0.02	ns
C18:3*n*-3	0.3^{a}	0.6^{b}	0.6^{b}	0.6^{b}	0.5^{b}	0.8^{c}	0.8^{c}	0.9^{c}	0.8^{c}	0.07	***	0.21	0.15	***
*c*9*t*11-CLA	0.3^{a}	0.4^{a}	0.4^{a}	0.6^{ab}	0.7^{b}	1.0^{c}	1.2^{c}	1.2^{c}	1.5^{d}	0.18	***	0.69	0.26	***
*c*9*c*11-CLA	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.02^{a}	0.04^{b}	0.05^{bc}	0.05^{bc}	0.07^{c}	0.02	***	0.04	0.02	***
tt-CLA	0.0^{a}	0.007^{ab}	0.0^{a}	0.0^{a}	0.006^{ab}	0.019^{b}	0.012^{ab}	0.011^{ab}	0.006^{ab}	0.008	**	0.01	0.009	***
C20:2*n*-6	0.015	0.011	0.005	0.005	0.005	0.002	0.003	0.000	0.000	0.007	+	−0.006	0.008	**
C22:0	0.06	0.07	0.07	0.07	0.06	0.07	0.08	0.07	0.07	0.01	ns	0	0.0011	ns
C20:3*n*-6	0.08^{c}	0.06^{ab}	0.06^{ab}	0.06^{a}	0.07^{bc}	0.06^{ab}	0.06^{ab}	0.06^{ab}	0.06^{a}	0.006	***	0	0.008	ns
C20:3*n*-3	0.002	0.009	0.010	0.002	0.003	0.007	0.007	0.011	0.002	0.006	*	−0.001	0.006	ns

Table III. Continued.

C20:4*n*-6	0.11^{c}	0.09^{ab}	0.09^{ab}	0.10^{ab}	0.10^{b}	0.08^{ab}	0.09^{ab}	0.08^{ab}	0.08^{a}	0.01	***	−0.01	0.01	***
C22:2*n*-6	0.01^{a}	0.04^{b}	0.05^{b}	0.04^{b}	0.03^{b}	0.04^{b}	0.03^{b}	0.04^{b}	0.03^{b}	0.008	***	0	0.011	ns
C20:5*n*-3	0.04^{a}	0.06^{b}	0.08^{c}	0.08^{c}	0.06^{b}	0.08^{c}	0.08^{c}	0.08^{c}	0.08^{c}	0.01	***	0.01	0.01	*
C22:4*n*-6	0.011	0.012	0.016	0.018	0.010	0.007	0.007	0.019	0.000	0.017	ns	−0.013	0.012	ns
C22:5*n*-3	0.072	0.089	0.085	0.085	0.082	0.093	0.092	0.088	0.094	0.014	ns	0.01	0.014	ns
C22:6*n*-3	0.001	0.003	0.002	0.003	0.001	0.018	0.000	0.000	0.001	0.015	ns	0.001	0.015	ns
Saturated FA	70.09^{d}	69.21^{d}	68.11^{cd}	66.81^{c}	64.72^{b}	62.15^{a}	60.98^{a}	60.91^{a}	60.81^{a}	1.48	***	−6.42	1.97	***
Linear odd short-chain FA[5]	0.18^{e}	0.15^{de}	0.13^{c}	0.09^{a}	0.14^{cd}	0.12^{bc}	0.10^{ab}	0.12^{bc}	0.10^{ab}	0.02	***	−0.01	0.03	ns
Linear odd medium-chain + BCFA[6]	3.92^{a}	4.37^{b}	4.89^{cd}	5.21^{d}	4.40^{b}	4.63^{bc}	4.87^{cd}	4.85^{cd}	4.99^{cd}	0.21	***	0.08	0.39	ns
Total *trans* FA[7]	2.30^{a}	2.12^{a}	2.33^{a}	2.68^{a}	3.96^{b}	5.21^{c}	5.56^{c}	5.96^{c}	6.94^{d}	0.62	***	3.11	0.99	***
SFAAI[8]	86.50^{c}	86.30^{c}	86.76^{c}	84.92^{c}	78.81^{b}	74.77^{a}	72.94^{a}	73.33^{a}	72.19^{a}	2.27	***	−11.66	2.90	***

[1] rSD = residual standard deviation.
[2] Statistical significance of feed variant effect.
[3] Statistical significance of seasonal effect: ns: $P > 0.10$; +: $P \leqslant 0.10$; *: $P \leqslant 0.05$; **: $P \leqslant 0.01$; ***: $P \leqslant 0.001$.
[4] Except for *t*16+*c*14-C18:1.
[5] Linear odd short-chain FA = C5:0 + C7:0 + C9:0 + C11:0.
[6] Linear odd medium-chain FA and branched-chain FA (BCFA) = C13:0 + C15:0 + C17:0 + *iso*-14 + *iso*-15 + *anteiso*-15 + *iso*-16 + *iso*-17 + *anteiso*-17 + *iso*-18.
[7] Total *trans* FA = sum of *trans* C18:1 + *trans* non-conjugated C18:2 + *tt*-CLA + *c*9*t*11-CLA.
[8] ASFAI = atherogenic saturated fatty acid index = C12:0 + 4 × C14:0 + C16:0, from Chilliard et al. [9].
[a,b,c,d,e] Means with different letters are different at $P < 0.05$ (Student-Newman-Keuls *t*-test).

correlated ($r = -0.56$) with permanent grassland forages, whereas *t*11 was positively correlated ($r = 0.88, P < 0.001$) with them. During winter, positive relationships were observed between *trans* isomers (except for *t*9) and the proportion of maize silage in the diet ($r = 0.47$ to 0.91, *t*10 and Fig. 3D). In contrast, the *t*11 isomer was negatively correlated with the proportion of maize silage in the diet ($r = -0.60$, $P < 0.01$). During this period, the *t*6+7+8- ($r = -0.77$, Fig. 3E), *t*10- ($r = -0.69$, Fig. 3F) and *t*12-C18:1 ($r = -0.70$) were negatively correlated with wrapped grass, whereas *t*11 was positively correlated ($r = 0.64$, $P < 0.01$).

On the scale of the year, only the *c*13 and *c*12 isomers of C18:1 were positively correlated with the proportion of maize silage in the diet ($r = 0.35$ and 0.81, respectively, Fig. 3C). During winter, all the *cis* isomers (except for *c*15) were positively correlated with the proportion of maize silage in the diet ($r = 0.55$ to 0.91). During the grazing period, only the *c*12 isomer was correlated with the proportion of maize silage ($r = 0.58, P < 0.001$, Fig. 3C).

4. DISCUSSION

4.1. Variability in milk fatty acid composition

Our study evidences a marked variability in the FA composition of milks that probably results from the broad diversity of the management systems, combined with seasonal changes in forage availability. However, the absence of seasonal variations in C4:0 and C6:0 percentages between grazing vs. winter feeding periods is consistent with the fact that these FA are synthesised in part by non-malonyl CoA mechanisms, i.e. not involving acetyl-CoA carboxylase, and thus less prone to regulation by dietary PUFA [8]. For other FA, we observed (Fig. 1 and Tab. III) that milks from the grazing period contained less saturated FA (C8:0 to C16:0), and more C18:0, *cis*- and *trans*-C18:1, *c*9*t*11-CLA, non-conjugated isomers of C18:2, and C18:3*n*-3 than those from the winter feeding period with grassland forages, in agreement with experimental data [7, 20] and with Swiss, German and French data obtained on cheeses or butters produced in summer and in winter [11, 27, 33, 39].

The variability in milk FA percentages in our study was similar to (C4:0, C16:0, *t*11-C18:1 and *c*9*t*11-CLA) or lower than that observed by Lucas et al. [34] in herd milks or cheeses collected in two different and distant geographical areas in France (North Alps and Massif Central). The variability in *t*11-C18:1 is more fully documented than that in other *cis* and *trans* isomers of C18:1. The range of the values of *t*11-C18:1 agrees with that observed in the studies of Precht and Molkentin [39] and Jahreis et al. [24] (0.4 to 4.4%) for German tanker milks from barn or pasture feeding. The variations in other *trans* C18:1 isomers were significant but narrower in our study, except for *t*13+14, which is the second major isomer and accounted for up to 0.58% of total FA during the grazing period, in agreement with Collomb et al. [12]. A similar large variation in CLA was found (0.3 to 1.1%) in tanker milks collected monthly during indoor or pasture feeding [24, 39] or in commercial butters from different producing areas in France [27]. Little information is available on the seasonal variations in non-conjugated *trans*-18:2 percentages. The variability in *t*11*c*15- and *c*9*t*13-18:2 in our study was of the same amplitude as that observed by Collomb and Bühler [11] and Precht and Molkentin [39] (0.04–0.68% and 0.07–0.32%, respectively) with Swiss and German tanker milks from barn or pasture feeding.

It is well established that milk fat *t*11-C18:1 is the substrate for endogenous *c*9*t*11-CLA synthesis via Δ-9 desaturase

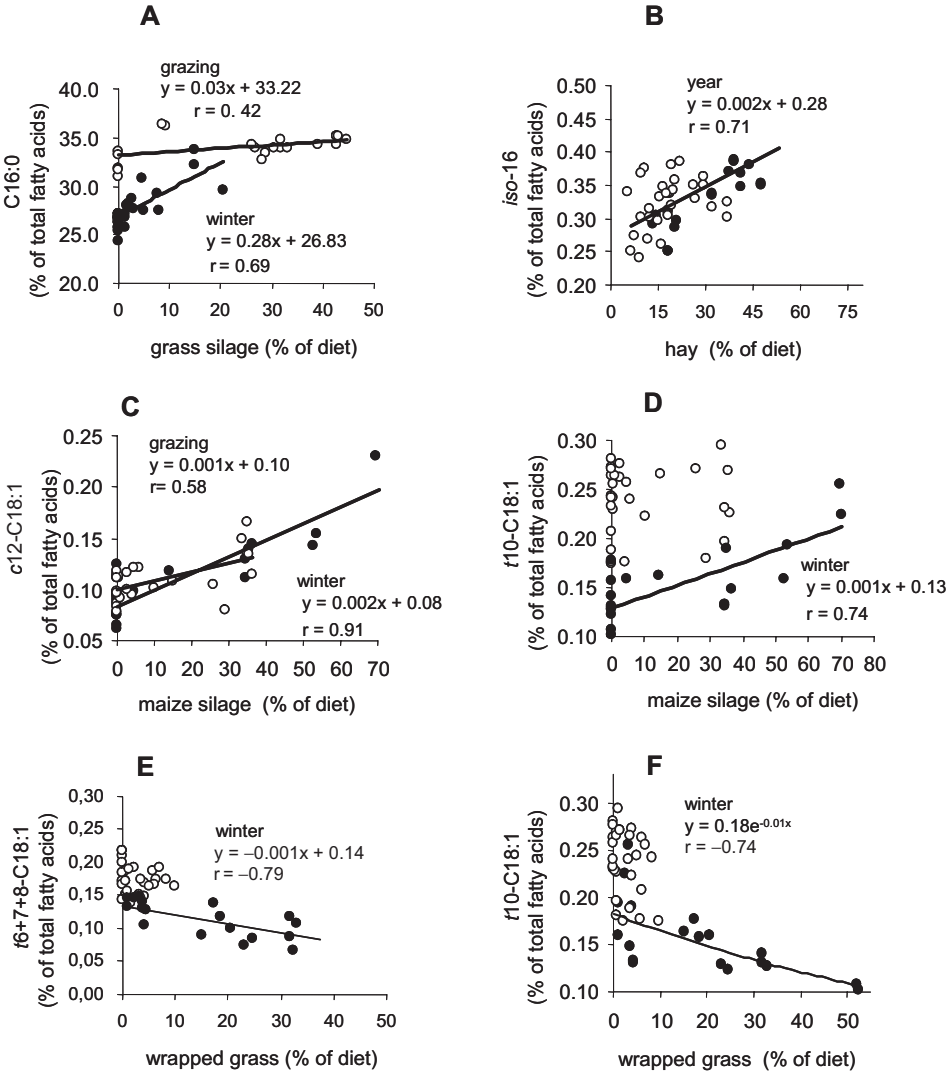

Figure 3. Relationships between *iso*-16, C16:0, *c*12-C18:1, *t*10-C18:1 and *t*6+7+8-C18:1 percentages in cow's milks from round tankers and proportions in the diet of grass silage, hay, maize silage or wrapped grass. • Winter feeding period, ○ grazing period.

[4, 5], and this was confirmed by the present high correlation coefficient between these two FA. In addition, the correlation coefficients between *c*9*t*11-CLA and *trans* isomers of C18:1 (*t*6+7+8 to *t*13+14) largely reflect the formation of these biohydrogenation intermediates during C18 FA metabolism in the rumen [30]. Similar positive coefficients between *c*9*t*11-CLA, *t*9-, *t*11-, *t*10- and total *trans* C18:1 were found with grass silage-based diets supplemented with marine or linseed oils [37]. The negative correlations between saturated FA and *c*9*t*11-CLA, *trans* isomers of C18:1,

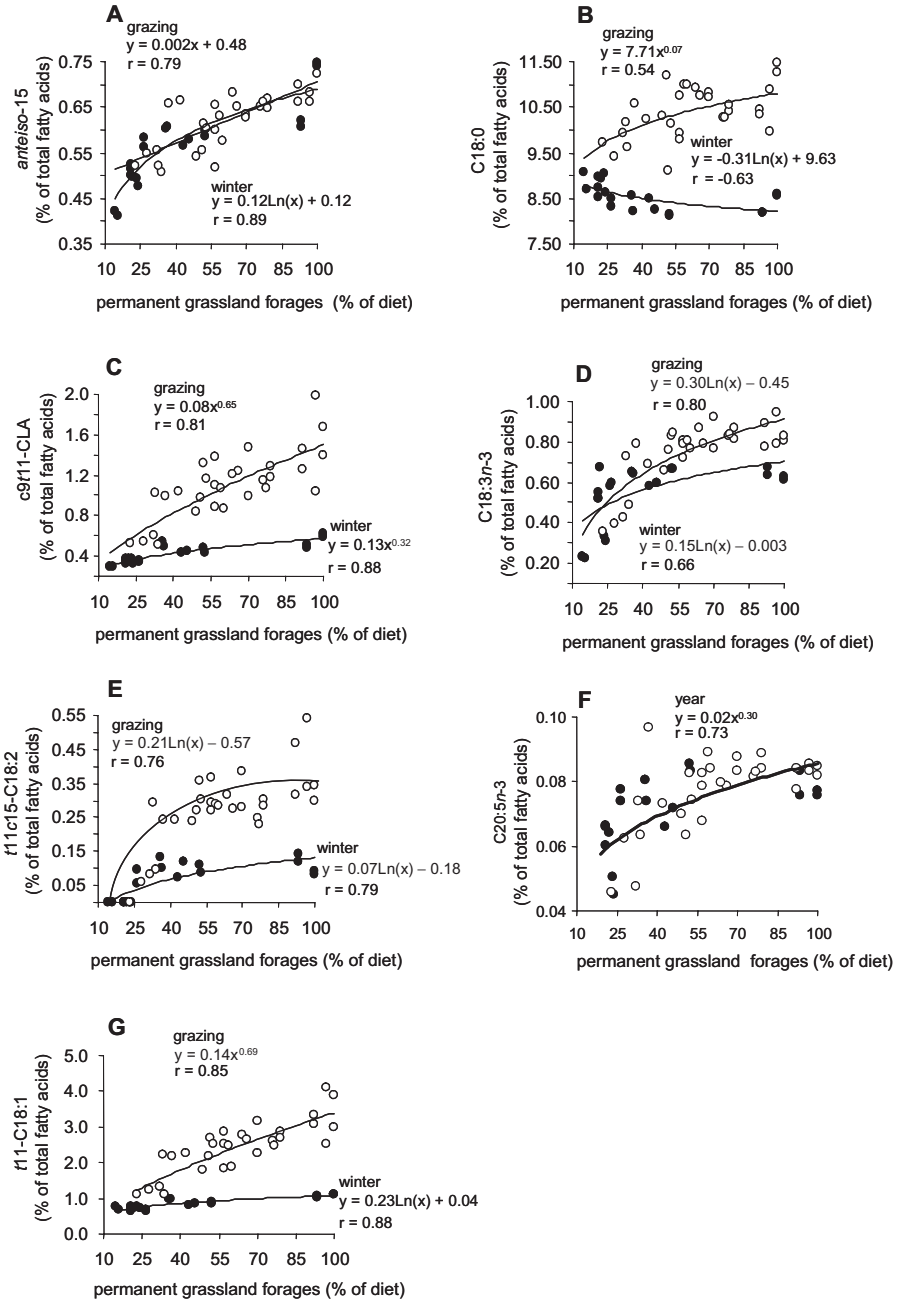

Figure 4. Relationships between *anteiso*-15, C18:0, *c*9*t*11-CLA, C18:3*n*-3, *t*11*c*15-C18:2, C20:5*n*-3 and *t*11-C18:1 percentages in cow's milks from the round tankers and proportions in the diet of permanent grassland forages (green or conserved). • Winter feeding period, ○ grazing period.

and C18:3*n*-3 are in agreement with Loor et al. [29] and may be explained by the fact that some long-chain FA, especially *trans*-C18:1 and/or C18:2 isomers, have a direct inhibitory effect on the de novo synthesis of the short- and medium-chain FA [4,5,8].

4.2. Effect of farm feeding and husbandry practices

The interpretation of the data is delicate because the different milk production conditions varied simultaneously. Caution must therefore be taken in assigning involvement to each factor. The influence of breed on the milk FA profile is minor, especially in the comparison between Holstein and Montbéliarde breeds, except for significant differences for C16:0 (+2.1 g·100 g^{-1} FA for Holstein vs. Montbéliarde breeds) and C18:0 (0.7 g·100 g^{-1} FA) contents [26]. The decrease in percentage of C16:0 from W1 vs. W4 variants could be explained partly by the lower proportion of the Holstein breed in the W4 variant.

Milk fat contents of *c*9*t*11-CLA, C18:3*n*-3 and *t*11*c*15-C18:2 were higher in G2–G5 groups (with a higher proportion of grazed grass in the diet) than in the G1 group. The C18:3*n*-3 intake may be higher during grazing [17, 31], especially for the young grass. Furthermore, either its putative higher nitrogen content (liable to increase the rate of lipolysis, the first step in the PUFA metabolism in the rumen) [18], or effects of fresh grass components (e.g. FA oxidation products being able to increase the biohydrogenation of C18:2*n*-6 and C18:3*n*-3 in vitro) [18] may have interacted to enhance the ruminal formation of PUFA biohydrogenation intermediates such as *t*11-C18:1, *t*13+14-C18:1 and *t*11*c*15-C18:2. It has indeed been demonstrated that increased *c*9*t*11-CLA content in milk fat from pasture-fed cows compared with cows fed total mixed ration is predominantly due to increased ruminal production of *t*11-C18:1 and its subsequent mammary Δ9-desaturation [25]. Furthermore, it has been shown that both duodenal flow of *t*13-C18:1 and *t*11*c*15-C18:2 [30] and milk fat concentrations of *t*13-C18:1, *t*11*c*15- and *c*9*t*13-C18:2 [14, 29, 30, 40] are enhanced in cows fed diets enriched with linseed oil, another dietary source of C18:3*n*-3. Earlier work in vitro presented evidence that *t*11*c*15-C18:2 was an intermediate of C18:3*n*-3 ruminal metabolism [23]. Also, the *c*9*t*13 isomer may be the reduction product of C18:3*n*-3 during ruminal biohydrogenation of C18:3*n*-3 [16]. However, close relationships between milk fat *t*13+14-C18:1 and *c*9*t*13-C18:2 content ($r = 0.70$, $n = 50$, $P < 0.001$) in the present study suggest that a proportion of *c*9*t*13-C18:2 secreted in milk is derived from endogenous conversion of *t*13-C18:1 via Δ9-desaturation in the mammary gland, in agreement with previous studies [30, 40] and with the very low duodenal flow of *c*9*t*13-C18:2 (< 0.01 g·d^{-1} [32]) compared with milk fat secretion (0.9 g·d^{-1} [29]) in cows consuming a hay-based diet.

The milk fat percentage of linear odd medium-chain FA and BCFA increased between G1 and G5 (Tab. III), with simultaneously increasing proportions of permanent grassland forages and/or pasture grazing, and decreasing proportions of maize silage in the diet. These findings are in line with the higher content of odd and BCFA in milk fat with grass-based diets [20], or with low-concentrate vs. high-concentrate diets [29]. Microbial synthesis of BCFA seems to be enhanced by diets rich in fibre, since increasing the proportion of dietary forage was closely related to the proportion of *anteiso*-15 in rumen bacteria [42]. Moreover, when maize silage was replaced with grass silage, milk *iso*-14 and *iso*-15 percentages increased [42]. In other respects, mammary synthesis from propionate and branched-chain VFA

may arise since odd and BCFA are strongly correlated with C4:0-C16:0 in lipid-supplemented cows [22].

The higher value of *t*10-C18:1 in W1 than in W2–W4 groups may be due to the higher level of starch in the diet, provided by the high proportion of maize silage. Indeed, low-fibre high-starch diets (vs. high-forage diets) may significantly increase the milk fat percentage of *t*10-C18:1 [4, 6, 40]. Moreover, during the grazing period, milk percentages of *t*10-C18:1 are similar to the values observed in W1, irrespective of the proportion of maize silage in the diet.

The milk percentages of C18:3*n*-3, *c*9*t*11-CLA and EPA also increased as a function of permanent grassland pasture and/or hay (Fig. 4), as reported by Dhiman et al. [19] and Couvreur et al. [15] for C18:3*n*-3 and *c*9*t*11-CLA. These data agree with several studies that have found elevated concentrations of C18:3*n*-3, *c*9*t*11-CLA and EPA in Alpine milks and dairy products [12, 18, 33]. Our farms at higher altitude had higher proportions of permanent grassland forages in the diet (i.e. W4 and G5 groups) and it can be supposed that these pastures presented a larger diversity of the botanical flora and induced milks richer in PUFA, as suggested by the correlation between milk PUFA and certain plant families and species in Alpine areas [13].

Few studies have compared milk EPA content from grass- or maize silage-based diets. In our study, milks from cows fed maize silage in the absence of fresh grass (corresponding to W1 and W2) were slightly but significantly lower in EPA than milks from cows fed grass-based diets. The higher milk content of EPA with grass-based diets may be due to the higher intake of C18:3*n*-3 with grass-based diets (Fig. 4F), which could putatively escape from ruminal biohydrogenation [20] and be partly converted endogenously into EPA, as has been shown in some mammalian species [36]. However, linseed oil supplementation increased milk C18:3*n*-3 but tended ($P = 0.15$) to decrease milk EPA yield [29]. Thus, factors other than an increase in C18:3*n*-3 intake are likely to be involved, although the milk concentration of these two FA are closely correlated in the present study (Fig. 2C).

We observed higher milk C18:0 content for G5 than for G1, corresponding to a higher proportion of permanent grassland forages in the diet. These data agree with previous ones [33]. In our study, energy expenditure in walking during the grazing period, when grass allowance may be decreasing, may have induced an increase in the proportion of stearic acid in milk due to body fat mobilisation [8]. Surprisingly, during winter, the opposite correlation was observed: the highest values in C18:0 content were obtained with simultaneously the lowest proportions of permanent grassland forages and diets rich in maize silage. This may be due to the greater intake of dietary C18:0, *c*9-C18:1 and C18:2*n*-6 provided by maize silage-based diets than by grass-based diets [18, 20].

Cows consuming mainly semi-mountain permanent grassland pasture (80% of the diet), and a small amount of concentrate (2.6 $kg \cdot d^{-1}$, Tab. I), produced milk with the highest concentration of *c*9*t*11-CLA (1.5% of total FA, G5 in Tab. III) and C18:3*n*-3 (0.8%). These levels are similar to those (1.3% *c*9*t*11-CLA and 1.0% C18:3*n*-3) observed in cows receiving a semi-mountain permanent grassland hay-based diet (65% of dry matter intake, plus 35% concentrate) supplemented with 3% linseed oil [29]. However, a very marked difference is that the milk from the cows receiving linseed oil contained 12.4% of total FA as *trans*-C18:1 and C18:2 isomers (including 7.9% of non-vaccenic/rumenic isomers), whereas in our G5 group this value was only 7.0% (including only 2.2% of non-vaccenic/rumenic isomers). Thus, the *trans* FA that are considered as most likely

to have pro-atherogenic effects [28] were 3.6 times less abundant in the milk from pasture-fed cows than in the milk from cows fed hay-based diets supplemented with linseed oil, despite their similar concentrations of rumenic acid and linolenic acid. This highlights the importance of assessing putative effects on human health of the different isomers of *trans*-FA, and deciding on rules for labelling all or only part of the *trans* FA in commercial dairy products.

5. CONCLUSIONS

The broad variability in FA composition of milks from the rounds observed in this study was closely linked to the variants of feeding and husbandry conditions prevailing in the geographical area studied. The milk fat from grazed grass had a higher proportion of total *trans* FA (including trans C18:1, non-conjugated C18:2 and *c*9*t*11-CLA) and total *cis* C18:1, whereas that from grass silage-based (and concentrate-supplemented) diets had more medium-chain saturated and monounsaturated FA (10 to 16 atoms). Milks from maize silage-based diets were richer in *c*12, *c*13, *t*6+7+8-, *t*10- and *t*12-C18:1, whereas those from grassland permanent forages were higher in BCFA and *n*-3 FA. During winter and grazing feeding periods, strong associations between the nature or mode of preservation of forages and some milk FA confirm the marked effect of nutritional factors on milk FA composition shown in experimental trials, and help to gain a better understanding of how they can interact in practical farm conditions. Given the variability in the FA composition of the tanker milks observed in this study and its links with the milk production conditions, it is possible to produce milks with an improved FA profile only by an appropriate selection of collecting rounds arriving at the dairy. Further issues need to be studied in experimental conditions such as the influence of the nature of grassland, and especially its botanical composition, to gain a more complete understanding of this variability, and particularly that in *trans* FA (C18:1 and both conjugated and non-conjugated C18:2).

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