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ORIGINAL PAPER

Mineral, vitamin A and fat composition of bulk milk related to European production conditions throughout the year

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Abstract In many Western countries, milk and dairy products provide both mineral and lipid fractions relevant to our health. The milk contents of these compounds are highly variable, and the identification of husbandry conditions leading to milks favourable for both mineral and lipid fractions is of interest. The aims of this study were to describe throughout the year the changes in bulk milk compounds and to relate them to husbandry practices. Ten compounds with nutritional requirements were selected: a minimum amount is required for Ca, Mg, P, Zn, vitamin A, n-3 fatty acids (FA), oleic acid and n-6 FA, and a maximum amount for *trans* FA and C12–14-16 FA. Milks and their associated husbandry practices were collected five times during 1 year

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from 74 groups of farms located in France, Norway, Slovakia and Slovenia. The Ca, Mg, P and Zn contents varied in different ways throughout the year and independently of each other. We showed the absence of milks with the highest contents in these four minerals simultaneously, but we identified groups of farms where milk had high content in Mg or in Zn. Positive links have been confirmed between grass-based-diets and milk n-3 FA content, maize silage-based-diets and n-6 FA content, and diets rich in concentrate and oleic acid content. These results showed that it was possible to identify husbandry conditions leading to milk favourable throughout the year for one or several compounds but not for all compounds considered in this study.

Keywords Micronutrients · Milk composition · Nutritional quality · Farming practises

1 Introduction

Improving the nutritional quality of milk is of concern to current producers, the dairy industry and consumers. Indeed, the dairy industry and retailers include increasing nutritional data in their public communication (Menard et al. 2012), and this trend is driven by the increasing number of well-known links between dairy products and human health (Visioli and Strata 2014). In many Western countries, the contribution of milk and dairy products in a balanced diet is recognized for the supply of both macro-constituents (proteins, fat and minerals) and micro-constituents (vitamins and trace elements). Considering the multiple compounds of interest in milk, the present study will explore, throughout the year, the changes in a set of nutritional relevant compounds: some minerals, fatty acids (FA) and vitamin A in relation with the associated husbandry conditions.

For minerals, the sole consumption of liquid milk in the European Union (170 $mL.day^{-1}.capita^{-1}$, IDF 2013) provides approximately 20–25 % of Ca and P and 10 % of Mg and Zn of the minimum daily amounts that are required for human metabolism (1000 mg of Ca, 750 mg of P, 260 mg of Mg and 7 mg of Zn) (WHO and FAO 2004). The contribution of dairy products to our fat consumption is also well-known. Some FA are considered desirable and a daily minimum intake is required, while others are usually consumed in excess and, therefore, a limited daily intake is recommended. The n-3 FA (mainly α-linolenic acid in milk and other minor long chain n-3 FA) limit metabolic syndrome and type 2 diabetes (Palmquist 2009), and thus a minimum daily consumption is required (2 g for C18:3n-3, the major n-3 FA) (FAO 2010). For oleic acid, the daily intake may range from 32 to 43 g (FAO 2010). The n-6 FA (mainly linoleic acid in milk) may activate adipose tissue development, and their daily intake may range from 4.3 to 8.5 g (FAO 2010). Among saturated FA (SFA), C12:0, C14:0 and C16:0 when consumed in excess may increase the risk of cardiovascular diseases (Shingfield et al. 2008). The daily intake of the sum of these three FA should not exceed 17 g (FAO 2010). The intake of *trans* FA from ruminant products does not seem to increase the risk of cardiovascular diseases in contrast to *trans* FA produced industrially (Gayet-Boyer et al. 2014). Nevertheless, for the FAO (2010), the daily *trans* FA intake (typically isomers of C18:1 *trans* derived from partially hydrogenated vegetable oils) should not exceed 1 % of the energy intake, i.e., 2.4 g. Based on an average daily consumption of 18 g per capita of dairy fat in the European Union (estimated from IDF, 2013) and on the fat composition reported by Coppa et al. (2013), dairy fat provides approximately 6 and 3 % of the recommendations for n-3 and n-6 FA, 9 % for oleic acid and up to 47 and 22 % for the sum of C12:0, C14:0, C16:0 and *trans* FA, respectively. Dairy fat also provides lipophilic vitamins, in particular one third of the vitamin A daily recommendation, which is 600 μg Eq retinol (WHO and FAO 2004).

The milk concentrations of all these compounds are highly variable throughout the year and vary according to the husbandry conditions. For dairy industries, there is an interest to characterize this variability and its variation factors. They are partially described for minerals and are well-known for FA and vitamin A. The milk mineral content varies mainly according to the lactation stage and the nutritional and health status of the dairy cows (Gaucheron 2005), whereas the vitamin A content is mainly affected by the nature of the forage, breed or calving period (Nozière et al. 2006). The concentrations of the various FA change according to the cow's diet and to a lesser extent to animal characteristics, such as breed and lactation stage (Chilliard et al. 2007). Among the mentioned factors, the cow's diet and lactation stage have a short-term effect on milk composition (<1 month). Therefore, changes in the farmer's practises throughout the year result in seasonal changes in bulk milk composition.

The variations in milk mineral contents or fat composition over 1 year have been described, in field studies, in herd milks (Rodriguez Rodriguez et al. 2001) or in dairy plant milks in Denmark (Poulsen et al. 2015), the Netherlands (Heck et al. 2009; van Valenberg et al. 2013), the USA (O'Donnell-Megaro et al. 2011) and the UK (Butler et al. 2011). The relationships between milk minerals and production systems have rarely been studied in on-farm conditions (Hurtaud et al. 2014). In contrast, many authors have reported changes throughout the year in milk fat composition (Butler et al. 2008; Ferlay et al. 2008; Collomb et al. 2008; Larsen et al. 2010; Borreani et al. 2013) or in fat-soluble vitamins (Agabriel et al. 2007; Ellis et al. 2007) according to the production systems. To the best of our knowledge, no study has investigated concomitantly minerals, FA and vitamin A in bulk milk and the associated husbandry conditions. Thus, the identification of husbandry conditions leading to the production of milks favourable for both mineral and lipid fractions throughout the year is the challenge of this study. Our strategy to achieve this aim is (1) to classify farms on the basis of the changes in mineral, vitamin A contents and fat composition in milk throughout the year and for each class, (2) to characterize the milk changes across the year and (3) to explain these changes according to the husbandry conditions.

2 Materials and methods

2.1 Study design

In our study, the bulk milk composition and the associated production conditions are studied at the scale of a group of farms (GF). In this manner, 74 GF were selected in France, Norway, Slovakia and Slovenia (Table 1) due to their large diversity of cow husbandries. Farms of each GF were geographically close and had similar milk production conditions. The different GF were located with a latitude range of 45.5 to 69.5°N and an altitude ranging from 40 to 1400 m a.s.l. The dairy cow diets were very

Table 1 Description of the study design

Country	France[a]	Norway	Slovakia	Slovenia[a]	Total
Number of GF	20	19	20	15	74
Number of farms	100	436	20[b]	144	700
Number of lactating cows	5463	9224	3881	2854	21,422
Breed of cows (number of GF)	Holstein (5) Tarentaise and Abondance (2) Holstein and Normande or Montbeliarde or Brown (13)	Norwegian Red (19)	Holstein (10) Slovak Spotted (10)	Holstein (3) Simmental (5) Brown (5) Brown and Simmental (2)	
Periods of milk sampling and survey with farmers in 2008 (Month-Date)					
Winter1	01–02 to 01–28	03–17 to 04–06	03–01	01–28 to 02–06	
Winter2	02–04 to 03–25	04–06 to 06–08	04–19	03–31 to 04–08	
Spring	04–28 to 06–25	05–26 to 06–20	06–09	05–16 to 06–05	
Summer	07–07 to 08–01	06–14 to 06–27	08–16	06–06 to 06–26	
Autumn	08–27 to 10–03	08–18 to 09–21	10–05	09–02 to 09–05	

GF group of farms

[a] GF constituted for this study

[b] Each farm consisted of one GF, considering the very high number of cows per farm in this country

diverse, ranging from diets based on grass (grass silage, hay or pasture according to the season) to diets including a large part of maize silage throughout the year. The proportion of concentrates ranged from nearly zero to more than 50 % of the dry matter intake (DMI). The annual milk yield ranged from approximately 3000 to more than 9000 kg.cow^{-1}.year^{-1}. The Holstein breed was the most common, with 22 GF having herds of more than 50 % Holstein cows. The Norwegian Red was the only breed that was present in Norway, while the Slovak Spotted and Holstein breeds were found in Slovakia (Table 1). Six other breeds (Brown, Montbeliarde, Simmental, Normande, Tarentaise and Abondance) were only present in France or in Slovenia.

Repeated measures—milk samplings and surveys—were performed on the 74 GF five times throughout 2008, over different periods depending on the country: twice during the wintering season, from January to March (winter1) and from February to May (winter2), and three times during the grazing season from May to June (spring), from June to July (summer) and mostly in September (autumn) (Table 1). Milk samples were collected from two or four successive milkings: In France and Slovenia, herd milk samples were equally pooled (which gives the same impact regardless of milk supply from individual farms), and in Norway and Slovakia, milk samples were directly collected in the collecting tanker truck (which results in proportional contribution to the bulk milk). Each of the 360 samples that were collected (10 missing samples) was then divided in 3 subsamples devoted to the analysis of minerals, vitamins and fatty acids. They were frozen without a preserving agent at −20 °C and stored until further analysis. Surveys were carried out to record the milk production conditions, particularly the cow’s diets, to be further linked to the milk characteristics. The national milk recording organizations provided milk yield according to the International Committee for Animal Recording standards.

2.2 Choice of milk compounds and analyses

The selected milk compounds were chosen among those used by the World Health Organization's published human nutritional requirements (WHO and FAO 2004; FAO 2010): Ca, Mg, P, Zn, vitamin A, the sum of n-3 FA (C18:3n-3, C20:5n-3, C22:5n-3, and C22:6n-3), oleic acid, the sum of n-6 FA (C18:2n-6, C18:3n-6, C20:2n-6, C20:3n-6, C20:4n-6 and C22:4n-6), the sum of C12:0, C14:0 and C16:0 (denoted C12–14-16), and finally, the sum of the *trans* FA (t9-C14:1; t11-C16:1; t4-, t5-, t6/7/8-, t9-, t10-, t11- and t12-C18:1; t9t12-C18:2; c9t13-C18:2; c9t14-C18:2; c9t12-C18:2; t9c12-C18:2; t11c15-C18:2; t11t13-conjugated linoleic acid (CLA), tt-CLA and c9t11-CLA). Minerals, vitamins and fatty acids were analysed by three laboratories respectively at the end of the collection period.

2.2.1 Minerals

Frozen milk samples were thawed overnight and homogenized by shaking and sonication at room temperature for 1 h. Closed microwave-assisted digestion (ETHOS 1600 microwave lab station, Milestone, Bergamo, Italy) was performed on 1000 g of a separate sample using 7 mL of 65 % nitric acid (HNO_3, SUPRAPUR, Merck, Darmstadt, Germany) plus 1 mL of 30 % hydrogen peroxide (H_2O_2, SUPRAPUR, Merck, Darmstadt, Germany) in PTFE vessels. Digested samples were diluted to 50 mL with MILLI-Q grade water. The concentrations of Ca, Mg and Zn in the digested milk samples were determined by flame atomic absorption spectroscopy (FAAS, AAnalyst 800, Perkin Elmer, Germany) as described in ISO 8070:(2007) and ISO 11813:(1998). The concentrations of P were determined by measuring the absorbance of a molybdenum-phosphate complex at 430.0 nm on a UV/VIS spectrometer (Cary 100, Varian, Mulgrave, Australia). For each mineral, the instrument was calibrated with five calibration solutions that were prepared by the dilution of commercial standard solutions (1000 $mg.L^{-1}$ Ca, 1000 $mg.L^{-1}$ Mg, 1000 $mg.L^{-1}$ Zn and 10,000 $mg.L^{-1}$ P, all CERTIPUR, Merck, Darmstadt, Germany). To control for contamination, a blank test was performed in each series. To confirm the accuracy of the analysis, a certified reference material, skim milk powder (CRM 063R Commission of the European Communities, Community Bureau of Reference, Brussels, Belgium), was also analysed in each series of digestions.

2.2.2 Vitamin A

Two volumes of milk were hydrolysed with three volumes of 12.5 M KOH/ethanol (2:1 *v*/*v*) for 25 min at 80 °C. BHT was added as an antioxidant. After cooling, retinol was extracted with hexane/toluene (1:1 *v*/*v*). The extract was then subjected to chromatographic analysis on an HP 1100 liquid chromatograph (Agilent Technologies, Palo Alta, CA, USA) equipped with an HP 1100 fluorescence detector, em: 325 nm ex: 480 nm. Vitamin A compounds were separated on a

4.6 mm × 100 mm normal phase silica column using 2 % 2-propanol in hexane as the mobile phase. A three-point calibration curve was used for quantification.

2.2.3 Fatty acids

Milk samples were lyophilised (ThermovacTM-20, Froilabo, Ozoir-la-Ferriere, France). The FA in lyophilised milk samples were methylated directly according to Ferlay et al. (2008). The FA methyl esters (FAMEs) were injected by auto-sampler into a Trace-GC 2000 series gas chromatograph that was equipped with a flame ionization detector (Thermo Finnigan, Les Ulis, France). The FAMEs 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 255 °C, and the detector temperature was maintained at 260 °C. The initial oven temperature was held at 70 °C for 1 min, increased to 100 °C at 5 °C.min^{-1} (held for 2 min) and then to 175 °C at 10 °C.min^{-1} (held for 42 min) and 5 °C.min^{-1} to a final temperature of 225 °C (held for 15 min). The carrier gas was hydrogen. The pressure was maintained constant during the analysis. Peaks were routinely identified by retention time comparisons with commercial authentic standards containing a mixture of FAMEs (NCP #463, Nu Check Prep, Elysian, USA; Supelco #37, Supelco, Bellefonte, PA, and O5632, Sigma).

2.3 Data analysis

2.3.1 Typology of GF according to the milk composition throughout the year

An innovative approach combining principal component analysis (PCA) and hierarchical cluster analysis (HCA) was performed to classify the 74 GF on the basis of the changes in the milk composition throughout the year (SPAD 7.4.56. Software for Data Mining, Data Analysis and Text Mining, Coheris SPAD, Suresnes, France). Using a standard PCA, the original set of 50 variables, consisting of 10 milk compounds measured 5 times, was transformed into a small number of uncorrelated variables known as principal components (PC) that retain as much of the information in the original variables as possible. HCA was applied to investigate nearness or similarities between GF, by maximizing intra-group homogeneity and intergroup diversity. The significance of the differences between classes for each of the 10 selected milk compounds was assessed using linear mixed model analysis (PROC MIXED, SAS, 2006–2010) in which the GF class was considered as a fixed factor, the sampling period as a repeated factor and the GF as a random factor. The interaction between GF class and sampling period was included in the model.

2.3.2 Characterization of cow husbandry conditions

Cow husbandries were described by the location (country and altitude), by lactating cows' characteristics (breed and daily milk yield) and by the diet fed at each sampling period. The diets were described using the database of the Norwegian Agricultural Authority or, in other countries, using the percentages of the different feeds as calculated according to the farmers' declarations. In France and Slovenia, an average diet was calculated during each period for each GF. When an ingredient was not quantified (pasture grass in particular), its quantity was estimated as the difference between the energy requirements of the herd and the energy that was provided by the known amounts of feed that were offered in the diet, assuming that the energy balance of the animals was zero. The maintenance requirements were estimated according to the average weight of each breed and milk production requirements according to the milk yield. The animal requirements and energy value of feeds were assigned according to the national feeding systems of each country. Among the variables describing the cow husbandry, the altitude (of the farmstead) and cow breed (proportions of the cows of the nine breeds) were considered constant over the year (i.e., 11 variables). One-way ANOVA was performed on these latter variables to determine the significance of the differences between the four GF classes. In contrast, the cow's diet was described five times a year by the proportion of hay, grass silage, maize silage and concentrate during the winter season and by the proportion of the same feeds plus pasture for the grazing season. The significance of the differences between the four GF classes for these diet variables and for the milk yield (average production/day/cow) was assessed using linear mixed model analysis (PROC MIXED, SAS, 2006–2010) in which the GF class was considered as a fixed factor, the sampling period as a repeated factor and the GF as a random factor. The interaction between GF class and sampling period was included in the model.

3 Results

3.1 Bulk milk composition and changes throughout the year

The average composition and variability of the 360 analysed milks are described in Table 2. The coefficient of variation (CV) was low (<10 %) for Mg and P and slightly higher for Ca, Zn and vitamin A (CV ranged from 13 to 18 %). Concerning FA, the variability of C12–14-16 and oleic acid was considerably lower (CV < 10 %) than that of the other FA that were considered (CV between 20 and 36 %).

The results of PCA are reported in Fig. 1. The n-3 FA at the five sampling periods were associated to *trans* FA during the grazing season on the negative part of PC1 (20.5 % of the total variability-TV) and were opposed to n-6 FA at the five sampling periods and Zn in the autumn on the positive part of PC1. PC2 (14.2 % TV) differentiated *trans* FA during the winter and oleic acid at the five sampling periods, from C12–14-16 at the five sampling periods. PC3 (9.2 % TV) mainly opposed Mg to n-6 FA at the five sampling periods, and PC4 (8.2 % TV) distinguished mainly Ca at the five sampling periods (data not shown). HCA, performed on 74 GF coordinates on the four first PC of the PCA (57.50 % TV), split the GF into four classes. Classes 1 and 4, with 13 and 24 GF, respectively, were projected on each side of PC1 and classes 2 and 3, with 23 and 14 GF, respectively, on each side of PC2 (Fig. 1). PC3 differentiated the classes 3 and 4 and PC4 differentiated the classes 2 and 4 (data not shown).

Significant differences in the contents ($P < 0.001$) between the four GF classes were observed for all 10 of the milk compounds (Fig. 2). The sampling period had a significant effect for all of the compounds, except for n-6 FA ($P < 0.05$), and the interaction between the class and the period had significant effects ($P < 0.01$) for all

Table 2 Bulk milk composition ($n = 360$ samples)

	Mean	Coefficient of variation (%)	Minimum	Maximum
Minerals, mg.kg^{-1}				
Calcium	1414	13.0	1022	2205
Magnesium	124	8.2	97	151
Phosphorus	903	5.0	758	1033
Zinc	4.1	16.3	2.5	6.5
Vitamin A, μmol.kg^{-1}	0.59	18.1	0.35	1.31
Fatty acids, g·100 g^{-1} of fat				
n-3 FA[a]	0.64	36.1	0.32	1.60
Oleic acid	19	9.6	15	25
n-6 FA[b]	1.74	20.1	0.94	3.49
C12–14-16[c]	45	7.5	36	54
trans FA[d]	3.84	34.1	1.82	8.68

[a] Sum of C18:3n-3, C20:5n-3, C22:5n-3, C22:6n-3

[b] Sum of C18:2n-6, C18:3n-6, C20:2n-6, C20:3n-6, C20:4n-6, C22:4n-6

[c] Sum of C12:0, C14:0, C16:0

[d] Sum of t9-C14:1, t11-C16:1, t4-, t5-, t6/7/8-, t9-, t10-, t11- and t12-C18:1, t9t12-C18:2, c9t13-C18:2, c9t14-C18:2, c9t12-C18:2, t9c12-C18:2, t11c15-C18:2, t11t13-conjugated linoleic acid (CLA), tt-CLA and c9t11-CLA

compounds, except for n-6 FA and oleic acid. Milks produced from class 1 had the highest content of n-3 FA throughout the year and the highest *trans* FA content in the grazing season. They had low oleic acid and n-6 FA contents throughout the year. The milks from class 1 presented the highest contrasting composition throughout the year, with the strongest decrease in C12–14-16 content and the highest increase in *trans* FA content when cows turned out to grazing. They had lower P and vitamin A contents than those of other classes and were richer in Ca, particularly in the spring. Milks from class 2 were relatively rich in C12–14-16 and had an intermediate content in n-3 FA. They were characterized by low mineral contents, particularly for Ca and Mg. The contents of the different compounds in milks from class 3 remained relatively constant throughout the year. The C12–14-16 contents were low, while oleic acid, Mg and vitamin A contents were globally high. Milks from class 4 contained the highest amount, throughout the year, in n-6 FA and Zn (except in winter1) as well as in C12–14-16 during the grazing season. Taking into account the overall mineral contents, the milks from class 4 presented the most balanced mineral contents.

3.2 Cow husbandry conditions associated with GF classes

The diet changes throughout the year according to the GF classes are described in Fig. 3. The diets were significantly different between the classes ($P < 0.005$,

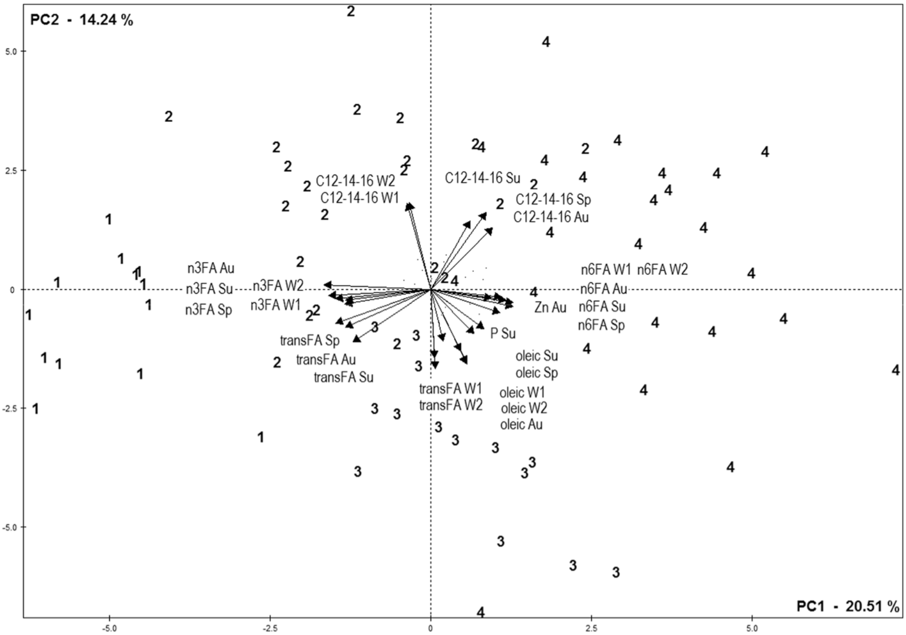

Fig. 1 Projection on the PC1-PC2 plane of the 74 groups of farms (GF) and the most correlated variables with the factor plan. GF is identified by the class number (1 to 4) to which it belongs. Most correlated variables with the factor plan: P (phosphorus), Zn (zinc), n3FA (sum of C18:3n-3, C20:5n-3, C22:5n-3, C22:6n-3), n6FA (sum of C18:2n-6, C18:3n-6, C20:2n-6, C20:3n-6, C20:4n-6, C22:4n-6), oleic (oleic acid), C12–14-16 (sum of C12:0, C14:0, C16:0), transFA (sum of t9-C14:1, t11-C16:1, t4-, t5-, t6/7/8-, t9-, t10-, t11- and t12-C18:1, t9t12-C18:2, c9t13-C18:2, c9t14-C18:2, c9t12-C18:2, t9c12-C18:2, t11c15-C18:2, t11t13-conjugated linoleic acid (CLA), tt-CLA and c9t11-CLA) - W1 (winter1), W2 (winter2), Sp (spring), Su (summer), and Au (autumn)

not shown), and as expected, the diets changed throughout the year ($P < 0.005$) with an interaction between the period and class for all feeds ($P < 0.005$). In class 1, the cow's diets were based on conserved grass in the winter (52 % hay, 20 % grass silage) and fresh grass in the grazing season (77 %), associated with concentrate (20 % in the winter and 12 % during the grazing season, respectively). In class 3, the cow's diets were also principally based on grass, grass silage during the winter (51 %) and fresh grass during the grazing season (55 %), and concentrate was distributed throughout the year (40 %). In classes 2 and 4, maize silage represented a significant proportion of forages in the diet: 20 % in the winter and 12 % in the grazing season for class 2 and 33 % in the winter and 25 % in the grazing season for class 4. The maize silage was mainly associated during the winter with grass silage (35 and 25 % for classes 2 and 4, respectively) and in the grazing season with fresh grass (45 and 23 % in classes 2 and 4, respectively). The concentrate was distributed throughout the year, 22 and 28 % on average for classes 2 and 4, respectively. The other production conditions are listed in Table 3. In class 1, GF were mainly located in France (70 %), where eight cow breeds were used, and the milk yield was significantly the lowest (17.1 kg per cow and per day). The GF from class 2 were widespread in Slovenia (48 %), France (26 %) and Norway (26 %). The

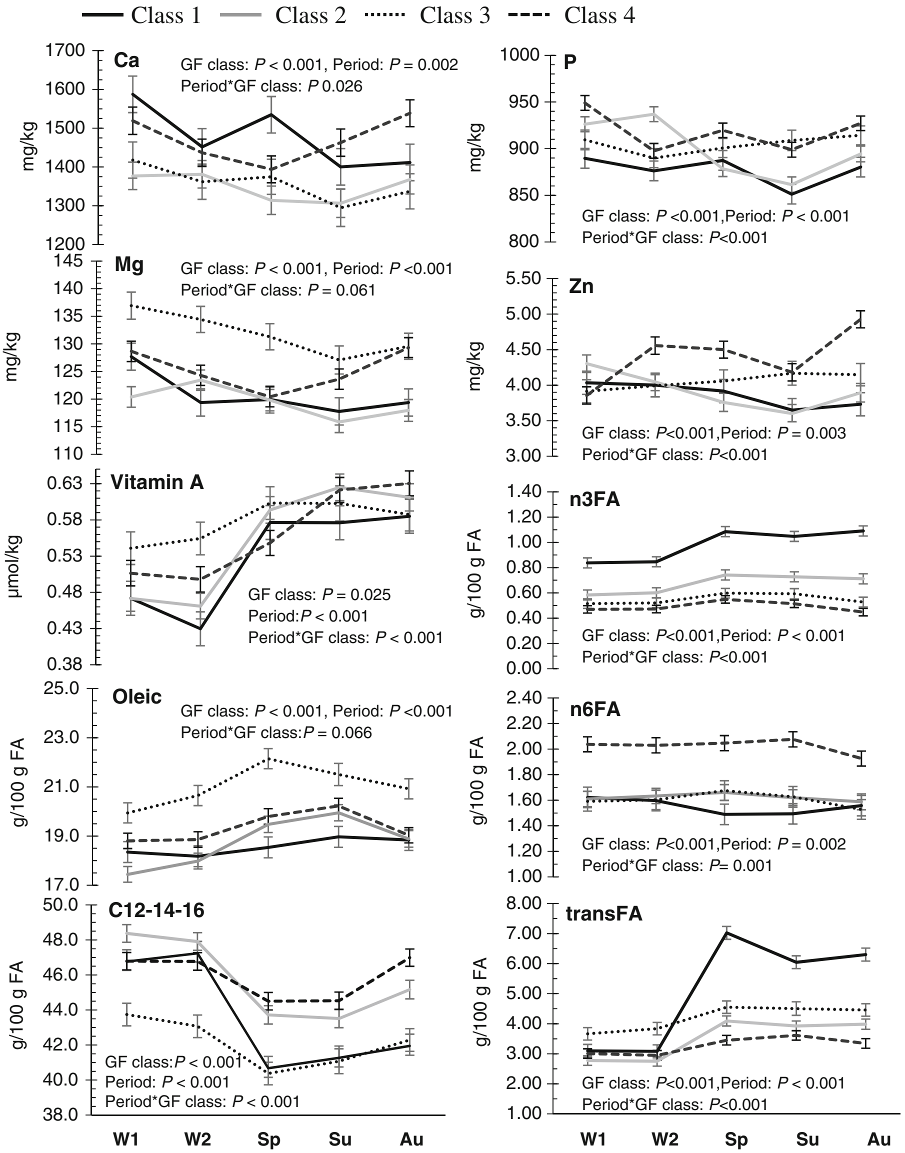

Fig. 2 Bulk milk composition throughout the year according to the group of farms classes and the sampling period. Sampling period: January–March (*W1*), February–May (*W2*), May–June (*Sp*), June–July (*Su*), and September (*Au*). Results are represented as adjusted mean and standards errors of means (*error bars*). The overlap of two errors bars indicates that values are not significantly different ($P > 0.05$). The significance of the effects of the GF class on milk composition, the sampling period and of the interaction between the period and the class is noted for each compound

major breeds were Holstein, Norwegian Red, Simmental and Brown, with an intermediate milk yield (20.2 kg per cow and per day). Class 3 was mainly represented by Norwegian farms with 86 % of Norwegian Red cattle. Slovakian GF constituted the main part (75 %) of class 4. Holstein was the major breed,

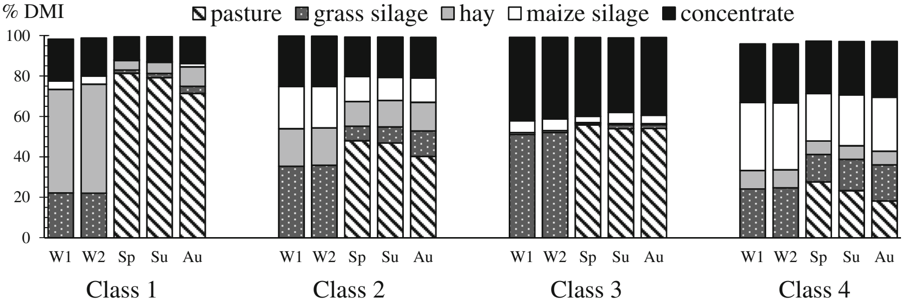

Fig. 3 Composition of cow's diet at milk sampling times according to the GF classes. *DMI* dry matter intake. Sampling period: January–March (*W1*), February–May (*W2*), May–June (*Sp*), June–July (Su), and September (*Au*)

followed by the Slovak Spotted (57 and 29 % of the total cows, respectively). The milk yield was the highest in both classes 3 and 4 (24.3 and 25 kg per cow and per day, respectively).

A slight variation of milk yield was recorded in the spring (turn out to grazing): There was an increase in classes 1 and 4 and a decrease in class 2. The milk yield remained stable throughout the year for class 3 (data not shown).

Table 3 Characterization of cow husbandry conditions on the four GF classes

	Class 1	Class 2	Class 3	Class 4	*P**
Number of GF	13	23	14	24	
Country, number of GF					
France	9	6	1	4	
Norway	0	6	12	1	
Slovakia	1	0	1	18	
Slovenia	3	11	0	1	
Altitude of farm, m a.s.l.	519 ^{a}	443 ^{a}	97 ^{b}	324 ^{a}	***
Major breeds of cows, % of total cows					
Holstein	13 ^{b}	29 ^{b}	7 ^{b}	57 ^{a}	***
Norwegian Red	0 ^{c}	26 ^{b}	86 ^{a}	4 ^{c}	***
Slovak Spotted	8 ^{b}	0 ^{b}	7 ^{b}	29 ^{a}	*
Brown	22 ^{a}	14 ^{ab}	0 ^{bc}	0 ^{c}	*
Montbéliarde	19 ^{a}	9 ^{ab}	0 ^{b}	2 ^{b}	*
Simmental	2 ^{b}	17 ^{a}	0 ^{b}	3 ^{b}	+
Normande	18 ^{a}	0 ^{b}	0 ^{b}	0 ^{b}	***
Tarentaise	10 ^{a}	0 ^{b}	0 ^{b}	0 ^{b}	*
Abondance	6 ^{a}	0 ^{b}	0 ^{b}	0 ^{b}	*
Milk yield, $kg.cow^{-1}.day^{-1}$	17.1 ^{c}	20.2 ^{b}	24.3 ^{a}	25.0 ^{a}	***

GF group of farms

*Statistical significance: means in the same row, followed by a different letter, are statistically different ($P < 0.05$) + $P < 0.01$, *$P < 0.05$, ***$P < 0.001$

4 Discussion

4.1 Milk mineral composition

The average mineral contents found in our study were within the range of those reported in herd milk by Rodriguez Rodriguez et al. (2001) and Lucas et al. (2006) and in dairy plant milk by Poulsen et al. (2015), except for Mg, which was 10 to 20 % higher in this study. The variability of the mineral contents of our bulk milk (pooled in transport tankers) was often close to that observed with herd milks in the literature. This variability highlights the possibility of distinguishing milks according to their mineral composition.

In our study, the four mineral contents in the different classes fluctuated according to different patterns throughout the year and did not show a general trend (Fig. 2). In contrast, Poulsen et al. (2015) reported that the contents of Ca, Mg and P were strongly associated and all showed similar seasonal variations, potentially due to their common metabolic pathway. These authors reported, for Ca, Mg and P, a positive correlation with milk protein content and noted the lowest values in the summer (July and August) and the highest values in winter (November to February). Similarly, Hurtaud et al. (2014) showed a common trend for the changes in Ca content in herd milk with the lowest values found in May and the highest in January. These seasonal patterns could be linked to block calving practises, but the data are not reported in these studies. In our case, the average lactation stage ranges from 158 to 202 days according to the sampling period and to the GF class (data not shown). This difference seems too limited to affect mineral content in milk (Gaucheron 2005). It is nevertheless noticeable that similarly to Poulsen et al. (2015), milk Ca and protein contents were positively correlated within the GF ($P < 0.001$, data not shown): It certainly reflects the functional link between Ca and the casein micelles. Hurtaud et al. (2014) suggested that the seasonal Ca content changes may be due to day length and hot temperature. Boudon et al. (2016), in an experimental study, demonstrated indeed that the Ca content significantly decreased during long and sunny days compared to short days. In our study, the average Ca content was also significantly lower in long vs. short days (1391 and 1470 mg.kg^{-1}, respectively), but we did not evidence that according to the GF latitude (highly variable in our design), the higher the difference between long and short days was, the greater the seasonal difference of milk Ca content was (data not shown). Considering that all the minerals in animal body and products come exclusively from the diet, the mineral content in milk could be also affected by the nutritional status of dairy cow (Gaucheron 2005). Nevertheless, little is known on the possible effect of minerals intake on milk composition. Maize is an extremely poor source of minerals compared to grass, and the mineral composition of the herbage differs greatly according to the species and their phenological stage. For example, the Ca content in plant is higher in legumes than in grasses (Pirhofer-Walzl et al. 2011). In our study, the richness in minerals of the milks from class 4 could be linked with a large proportion of clover and alfalfa in the forage and concentrate distributed in GF from Slovakia. It can also be noted that on pasture, a high content of clover may change the dynamics of milk minerals compared to grass only swards because the proportion of clover changes substantially throughout the season, being highest in autumn. Moreover, the high Mg contents of milks from class 3 could be associated with the abundance in herbs in half of the GF, as herbs are particularly rich in this mineral. The low variability of the mineral contents in milks from class 3 could also be related to the constant and significant amount of commercial concentrates, which generally are enriched with minerals. In our study, it is nevertheless difficult to elucidate the effect of diet on milk minerals because of probable confounded effects with mineral supplementation, fertilization practises, pedo-climatic context and cow breed. These different effects cannot be segregated with our dataset. A breed effect on the milk mineral content has also been reported (Poulsen et al. 2015). In our study, in the sole situation where two breeds were reared in the same country (Slovakia) with similar diets, we did not found any difference between Holstein and Slovak Spotted breeds on the mineral contents in milk.

4.2 Milk vitamin A composition

The vitamin A contents that were measured in our study were approximately 40 % lower than the average value reported by Lucas et al. (2006) and Agabriel et al. (2007) in bulk milk, and within the range of those reported by Martin et al. (2002) in an experimental study. The observed annual change in vitamin A content is consistent with the literature: It increased between the winter and grazing periods, simultaneously with the increasing proportions of fresh herbage in the diet (Figs. 2 and 3). This high milk content in vitamin A is explained by the higher β-carotene content in fresh grass than in all conserved forages (Nozière et al. 2006). Indeed, the β-carotene acts as the main precursor of vitamin A for ruminants (Nozière et al. 2006). During the winter season, we observed a large diversity in the vitamin A content in milk. The highest contents were associated with diets including a major proportion of both grass silage and concentrate (Figs. 2 and 3, class 3). The grass silage contains three times more carotenoids than hay which increase vitamin A in milk. In addition, commercial concentrate is very often enriched with vitamin A, which is directly transferred into the milk (Nozière et al. 2006). On the opposite, the lowest vitamin A contents were associated with diets including a large part of hay, which contain very few carotenoids and a low proportion of concentrate (Figs. 2 and 3, class 1).

4.3 Milk fat composition

The effect of diet on FA concentrations in milk has been clearly shown under experimental conditions (Chilliard et al. 2007; Shingfield et al. 2013) and validated on commercial farms (Ferlay et al. 2008; Slots et al. 2009; Borreani et al. 2013). Coppa et al. (2013) have even predicted bulk milk FA composition using data describing farming practises via on-farm surveys. Our results, at the level of a large-scale design, are completely consistent with these publications.

In our study, the average content of C12–14-16 was consistent with those of Ferlay et al. (2008) and Heck et al. (2009), higher than those observed by Collomb et al. (2008) and O'Donnell-Megaro et al. (2011) and lower than that shown by Butler et al. (2011). The lower content of C12–14-16 reported by Collomb et al. (2008) (34.9 and 41.1 g × 100 g^{-1} fat in the summer and in the winter, respectively) could be explained by the fact that cows were fed diets high in grass (74 % of fresh grass in the summer and 69 % of hay in the winter) from the Swiss mountains. Feeding the cows with these diets, which are rich in polyunsaturated fatty acids (PUFA), explains the decrease in these SFA, via an inhibition of de novo mammary synthesis of all C12:0, C14:0 and half of C16:0 by *trans* FA resulting from the rumen biohydrogenation of PUFA ingested (Chilliard et al. 2007). This mechanism could explain the low C12–14-16 content observed in our study during summer in class 1, where the proportion of grazed grass is very high (Figs. 2 and 3). Moreover, O’Donnell-Megaro et al. (2011) associated the low content of C12–14-16 they found in dairy plant milk (40.5 g × $100g^{-1}$ fat) to the low forage diet and the routinely use of lipid supplement and by-product in the USA. The unsaturated FA from the latter feedstuffs and the reduction in forage intake depress the de novo mammary lipogenesis, thereby reducing the SFA content in milk (Chilliard et al. 2007). Similar hypotheses could explain the low values of C12–14-16 found in our study for the class 3 mainly made of Norwegian farms where cow diets included a high and constant proportion of commercial concentrate (40 %) comprising fat added from different rape products (70–75 % of the fat added). In the UK, Butler et al. (2011) proposed that the high C12–14-16 level (50.3 g × $100g^{-1}$ fat) in some of their dairy plant milks could be associated with the wide use of commercial feed products, including palm oil, a practice known to increase the C16:0 concentration in milk (Larsen et al. 2010).

The average values of oleic acid that were observed in our study were higher than the average values that were observed by Collomb et al. (2008) and Heck et al. (2009) (+1.9 and +1.4 g × $100g^{-1}$ fat, respectively). Our findings were consistent with those of Ferlay et al. (2008), and they were lower than those observed by Butler et al. (2011) in conventional milks collected in retail outlets and particularly by O’Donnell-Megaro et al. (2011) (−2.9 and −4.6 g × 100 g^{-1} fat, respectively), who linked this high milk oleic acid content with dietary lipid supplements. Moreover, Glasser et al. (2008) have shown that milk oleic content increased significantly with lipid supplements rich in long-chain unsaturated FA (with atoms of carbon ≥18). The high milk concentration of oleic acid shown by O’Donnell-Megaro et al. (2011) could be explained by the fact that this FA is an intermediate of ruminal biohydrogenation of unsaturated FA from dietary lipid supplements. Milk oleic acid results also from the mammary Δ-9 desaturation of stearic acid which is the end-product of the ruminal biohydrogenation of dietary lipids (Chilliard et al. 2007). In our study, the incorporation of lipids from rape (rich in oleic acid) in Norwegian commercial concentrates is probably the main reason why milk oleic acid content is constantly the highest across the year (class 3).

The n-3 FA average contents in milk from our study were comparable to the annual average values that were observed by Butler et al. (2011) and van Valenberg et al. (2013). In contrast, Ferlay et al. (2008) and Collomb et al. (2008) reported a higher n-3 FA content (+0.19 and +0.45 g × 100 g^{-1} fat, respectively) with grass-based diets. Similarly, the highest n-3 FA contents in milk observed all over the year for class 1 was associated with the highest proportion of grass in the diet (hay and grass silage during winter and pasture during summer) and low amount of concentrate. Grass-based diets are rich in C18:3 n-3 and therefore are known to increase the n-3 FA milk content up to five times when fresh grass is compared to maize-silage diet (Ferlay et al. 2006). In addition, Collomb et al. (2008) also suggested that the mountain location could reinforce the effect of grass due to a combined effect of altitude via cow hypoxia and of botanical diversity of the permanent grassland. The latter may provide plant secondary compounds (terpenoids and phenolic compounds) able to limit rumen biohydrogenation and therefore increase milk C18:3 n-3 content. Indeed, in this study, the GF located at the highest altitude are all included in class 1.

The milk in our study had n-6 FA contents that were comparable to those observed by Ferlay et al. (2008) and van Valenberg et al. (2013) and lower than those that were found by Butler et al. (2011) (−0.3 g·*100 g^{-1} fat). These latter milks were produced in conventional farms where cows could have been fed high-maize silage diets. In our study, the highest n-6 FA contents were associated with diets including the highest part of maize silage (Figs. 2 and 3, class 4). This fodder is rich in C18:2 n-6 (nearly 1.5 % DM) and therefore increases milk C18:2 n-6 content (Chilliard et al. 2007). Moreover, Slots et al. (2009) have highlighted that the n-6 FA milk contents were positively linked to commercial concentrate mix. In contrast to our results, they showed no correlation between the maize silage intake (10 to 30 % of DMI) and the n-6 FA content in the milk.

The average *trans* FA content in our milks was equivalent to that reported by Ferlay et al. (2008). No other comparison could be made, because the method of calculation for the *trans* FA sum was different according to the authors and all of the data were not available. Considering the changes in *trans* FA content throughout the year, our results agree with the literature, i.e., a strong elevation in milk *trans* FA content observed in class 1 when cows grazed pasture (Butler et al. 2008; Ferlay et al. 2008; Collomb et al. 2008; Larsen et al. 2010). The richness in C18:3 n-3 of pasture could explain the ruminal production of *trans* FA from ruminal biohydrogenation of C18:3 n-3 and then the high milk content of *trans* FA. The higher levels of *trans* FA during grazing than winter season for class 1 could also be reinforced by the phenological stage of the grass (more digestible lush grazed grass versus mature conserved forage) that influences the ruminal biohydrogenation pathway of PUFA (Chilliard et al. 2007). In addition, we observed that *trans* FA content did not increase with pasture diets including a large part of concentrate (Figs. 2 and 3, class 3) and therefore a least part of grazed grass in the diet.

Moreover, we have highlighted three out of four classes of GF mainly gathering farms located in the same country. This result could indicate that specific production conditions exist within the country and affect the milk characteristics, as shown by Gaspardo et al. (2010), with neighbouring Italian and Slovenian farms having similar practises.

4.4 Nutritional interest of bulk milk according to GF classes

For minerals, in order to compare the nutritional interest of our milks according to the RNI, we only considered the average daily liquid milk consumption in the European Union, i.e., 170 mL in 2012 (IDF 2013). Indeed, it was difficult to estimate the total mineral intake from all dairy products then anyway, when milk is transformed, a variable fraction of minerals moves from milk to whey according to the cheese-making process (range from 1 to 10 for Ca, for example) (Feinberg et al. 1987). As the mineral content of most dairy products is driven by the process, the variability of the mineral content of milk affects human mineral intake mainly through the consumption of liquid and fermented milk like yoghurt. The total fat dairy intake can be estimated more easily because most of the processes do not modify the fat composition (Lucas et al. 2006). In Europe, it approximates 18 g.day^{-1} and per capita (IDF 2013).

According to the GF classes, the average supply of the RNI (WHO and FAO 2004; FAO 2010) ranged from 23.6 % (class 2) to 25.8 % (classes 1 and 4) for Ca, from 18.4 % (class 4) to 28.7 % (class 1) for *trans* FA, from 4.4 % (class 4) to 8.8 % (class 1) for n-3 FA and from 44.6 % (class 3) to 48.6 % (class 4) for C12–14-16. On the opposite, the average supplies of the RNI for P, Mg, Zn, vitamin A, n-6 FA, and oleic were almost similar for the different classes (maximum difference about 1 %). Therefore, the proposed GF classification seems meaningful from a nutritional point of view only for Ca, n-3 FA, C12–14-16 and *trans* FA. Considering these four compounds, we underline the absence of milks with the highest nutritional interest in order to reduce simultaneously the *trans* FA and C12–14-16 intake and to increase the n-3 FA and Ca intake. For example, the choice of milks produced by farms from class 1 seems relevant to increase the intake of Ca and n-3 FA throughout the year and to reduce the C12–14-16 intake during the pasture season, but meanwhile, it dramatically increases the *trans* FA intake. We have nevertheless to consider that in class 1, *trans* FA mainly consist in vaccenic acid (data not shown) which is converted to rumenic acid by desaturation in human metabolism. Rumenic acid is only found in ruminant fat and has been linked to many potential health benefits. The choice of milks from class 3 could be a lever to reduce the C12–14-16 intake throughout the year, but it is irrelevant for Ca and n-3 FA supplies. Finally, the potential nutritional impact of the milk composition differences found here needs to be evaluated in the context of the overall diet. For heavy consumers of dairy product, the effects previously highlighted could be reinforced.

5 Conclusion

Based on a wide-ranging framework, this study investigated changes throughout the year in the contents of bulk milk compounds with nutritional requirements. The approach focused on a set of minerals, fat-soluble vitamins and FA with the associated husbandry conditions. We observed that the Ca, Mg, P and Zn contents evolved in different ways throughout the year, and they evolved independently of each other. We showed the absence of milks with the highest contents in these four minerals simultaneously, except in the autumn. Moreover, we identified GF where milk had high content in Mg or in Zn throughout the year. We confirmed that the FA profile and the vitamin A contents remained strongly associated with the diet composition. We also highlighted that no GF produce milks with a significant contribution to the five FA requirements, independent of the sampling dates. Our results suggested that groups of farms could be selected to collect milks with some specific characteristics throughout the year or during some periods. Throughout the year, grass-based diets remained the best method to produce milk with high n-3 FA content. Maize silage-based diets resulted in milk with high n-6 FA content, while those rich in commercial concentrate including rape induced an increase in oleic acid content in milk. During the outdoor period, grazing remained clearly the most effective means with a low C12–14-16 FA content in milk, accompanied by a concomitant increase in *trans* FA. These FA did not increase with diets rich in concentrate. During the indoor period, diet with both grass silage and commercial concentrate led to high milk vitamin A content. Thus, in practice, it is possible for dairy industry to take advantage of the location collection area and the diversity of the farming systems, to segment the dairy products using individual specific milk compounds. Nevertheless, it was not possible to identify milks relevant to our health for both mineral and lipid fractions throughout the year.

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Compliance with ethical standards

Conflict of interest Chantal Chassaing, Cécile Sibra, Jože Verbič, Odd Magne Harstad, Jaroslav Golecký, Bruno Martin, Anne Ferlay, Isabelle Constant, Carole Delavaud, Catherine Hurtaud, Vida Žnidaršič Pongrac and Claire Agabriel declare no conflict of interest.

Compliance with ethics guidelines This article does not contain any studies with human or animal subjects performed by any of the authors.

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