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Potential of milk fatty acid composition to predict diet composition and authenticate feeding systems and altitude origin of European bulk milk

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ABSTRACT

The aims of this work were to elucidate the potential of using milk fatty acid (FA) concentration to predict cow diet composition and altitude of bulk milk collected in 10 different European countries and to authenticate cow-feeding systems and altitude of the production area using a data set of 1,248 bulk cow milk samples and associated farm records. The predictions based on FA for cow diet composition were excellent for the proportions of fresh herbage [coefficient of determination (R^2) = 0.81], good for hay, total herbage-derived forages, and total preserved forages ($R^2 > 0.73$), intermediate for corn silage and grass silage ($R^2 > 0.62$), and poor for concentrates ($R^2 < 0.51$) in the cow diet. Milk samples were assigned to groups according to feeding system, level of concentrate supplementation, and altitude origin. Milk FA composition successfully authenticated cow-feeding systems dominated by a main forage (>93% of samples correctly classified), but the presence of mixed diets reduced the discrimination. Altitude prediction reliability was intermediate ($R^2 < 0.62$). Milk FA composition was not able to authenticate concentrate supplementation level in the diet (<58% of samples correctly classified). Similarly, the altitude origin was not successfully authenticated by milk FA composition (<76% of samples correctly classified). The potential of milk FA composition to authenticate cow feeding was confirmed using a data set representative of the diversity of European production conditions.

Key words: milk fatty acid, feeding system, altitude, authentication, dairy cow

INTRODUCTION

Consumers are becoming increasingly interested in the authenticity of food. For dairy products, this is especially true when consumers purchase expensive certified and high-added-value products, such as organic, protected designation of origin, or protected geographical indication products (Capuano et al., 2013). These products are characterized by specification protocols related to agronomic practices and animal diet composition (nature of forages, concentrate supplementation limits) or can be produced in restricted territories. In addition to traditional traceability systems based on documents, increasing need exists for analytical tools applicable to products enabling the authentication of specification protocols (Engel et al., 2007).

Several studies have aimed to develop reliable analytical methods applicable to dairy products for the authentication of animal diets or geographical origin (Prache et al., 2007). Plant biomarkers found in dairy products, such as terpenes and phenolic compounds, seem to be valuable for the authentication of pasture-derived milk and cheeses, especially those produced on highly biodiversified upland pastures (Tornambé et al., 2006; Besle et al., 2010; Coppa et al., 2011). However, these compounds differ widely according to the method of herbage conservation (fresh grass, hay, or silage), botanical composition, and phenological stage (Cornu et al., 2001; Sangwan et al., 2001; Reynaud et al., 2010). Other plant metabolites, such as carotenoids, have been successfully used to distinguish pasture- from cereal-derived dairy products (Butler et al., 2008; Slots et al., 2009; Stergiadis et al., 2012). However, carotenoids were less valuable to authenticate cow diets in the case of mixed diets including small proportions of fresh herbage or based on different conserved forages (Nozière et

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al., 2006; Slots et al., 2009; Stergiadis et al., 2012). Carotenoids also seem to be unreliable for the authentication of upland dairy products (Engel et al., 2007). As part of the fats, Baars et al. (2012) showed that the combination of phytanic acid concentration and diastereoisomers ratio could discriminate between intake of fresh grass and silages of maize plus concentrates. Capuano et al. (2014) used phytanic acid and pristinic acid to identify the ration of the cows in terms of fresh grass intake and showed that the diastereoisomers ratio was useful to discriminate in the fresh grass intake, whereas Kaffarnik et al. (2014) combined phytanic acid measurements with carbon isotopes to discriminate different feeding origins. The ratios between milk-stable isotopes of N, H, or O have been successfully used to authenticate the geographical region (Manca et al., 2001; Renou et al., 2004; Ehtesham et al., 2013) or altitude origin (Engel et al., 2007) of dairy products. However, stable isotopes seem to be less efficient for the authentication of animal feeding (Renou et al., 2004; Engel et al., 2007). Recently proposed global approaches based on milk infrared spectra analysis (**IR**) are promising rapid and cheap methods to authenticate cow feeding, but failed to identify production altitude (Coppa et al., 2012b; Valenti et al., 2013).

Among the different analytical tools for the authentication of dairy products, milk FA composition was found to be the most efficient method to provide precise information about cow feeding and altitude origin (Engel et al., 2007). Differences in milk FA composition related to diet composition are now well established for controlled conditions [i.e., the reviews of Chilliard et al. (2007) and Shingfield et al. (2013)] and have been confirmed on farm (Borreani et al., 2013; Coppa et al., 2013; Hurtaud et al., 2014; Kusche et al., 2014). Milk from grazing herds is characterized by higher concentrations of PUFA and MUFA, C18:3n-3, C18:1 *trans*-11, CLA, and branched-chain FA, whereas cows fed hay-based diets produce milk rich in C18:3n-3 but with lower CLA content compared with grazing cows. Corn silage and concentrates in the cow diet increased the saturated FA and C18:2n-6 and reduced the C18:3n-3 and CLA concentrations in milk (Dewhurst et al., 2006; Chilliard et al., 2007; Shingfield et al., 2013). Furthermore, Gaspardo et al. (2010) showed differences in milk FA composition according to different geographical region (northeast Italy and Slovenia). Similar results were observed by Kraft et al. (2003) for CLA isomer concentrations in milk from Swiss regions at different altitudes. Engel et al. (2007) proposed some ratios between FA to authenticate altitude origin and cow diet of milk from central France. Upland milk showed higher C18:3n-3, branched-chain FA, and CLA *cis*-9,*trans*-11, and lower C16:0 and saturated FA concentrations than lowland milk (Kraft et al., 2003; Engel et al., 2007). However, all the studies proposing FA to authenticate the geographical origin of milk compared groups of samples differing also for farming practices and cow feeding. Thus, it remains uncertain whether the results were due to the direct effect of altitude or more likely to the cow-feeding system, pasture botanical composition, and breed of cows kept that usually change along the altitude gradient. Furthermore, most of the literature aiming to authenticate cow feeding or altitude origin have been made on a small territorial scale. As farming practices and cow diets vary widely according to the country and agronomic context, literature findings should be validated over a larger geographical area. The aims of the current study were (1) to predict cow diet composition and altitude from FA concentrations in bulk milk collected in different European countries and (2) to authenticate cow-feeding systems and altitude of production areas from which milks are derived.

MATERIALS AND METHODS

Data Collection

The FA profiles of 1,248 bulk cow milk and their related farming practices were compiled from a selection of 20 published or unpublished studies carried out from 2000 to 2010 in 10 different European countries: Czech Republic, Denmark, France, Germany, Italy, Norway, Slovakia, Slovenia, Sweden, and the Netherlands. It included milk collected on commercial farms at between 44 to 69°N latitude, from sea level to 2,100 m in altitude, from 13 different cow breeds, and in all seasons. The detailed composition of this data set is described in Coppa et al. (2013).

FA Composition

Milk FA analyses were performed by 5 different laboratories over the 2001 to 2010 period using gas-chromatographic methods. The analytical methods used are given in Coppa et al. (2013).

Due to the heterogeneity among experiments in FA identification, a simplified FA profile was selected with the aim of finding a trade-off between the number of FA studied and the number of samples for which these FA were reported, as described in Coppa et al. (2013). In the earlier experiments where C18:1 *trans*-10 and C18:1 *trans*-11 were co-eluted and not separated and only C18:1 *trans*-11 was reported, it was considered as the sum of C18:1 *trans*-10 + *trans*-11. Separate proportions of C18:1 *trans*-10 and C18:1 *trans*-11 reported in 592 samples were used to perform a separate calibration on this smaller sample subset. Similarly, C18:1 *cis*-

9 reported in these studies was assumed to be the sum of C18:1 *cis*-9 + *trans*-13.

Production Conditions

Data related to production conditions collected at each milk sampling via on-farm surveys included herd characteristics, diet composition for lactating cows, and altitude of the farm (or grazed plots), as described by Coppa et al. (2013). During surveys, the quantities of the different feedstuffs (grass silage, hay, corn silage, concentrates, total conserved forages, and total herbage-derived forages) provided to lactating cows were estimated directly by the farmers and expressed as the proportion of each feedstuff in cow diet on a DM basis. Fresh herbage intake at pasture was estimated as described by Coppa et al. (2013).

Sample Categorization

In authenticating feeding systems and altitude, all milk samples were categorized according to (1) feeding system, (2) conserved forage offered, (3) level of concentrate supplementation, and (4) production altitude, as explained herein with details summarized in Table 1.

(1) Feeding Systems. Milk derived from diets with over the 50% of DM from forage were assigned as fresh herbage (**FH >50**), grass silage (**GS >50**), hay (**H >50**), or corn silage (**CS >50**) based on the dominant forage. Milk samples derived from diets in which none of the forages reached at least half of the DM proportion were assigned to the mixed feeding system (**MIX**).

(2) Conserved Forage-Based Feeding Systems. As the proportion of fresh herbage in cow diet was found to affect milk FA composition much more than the proportion of each of the preserved forages (Chilliard et al., 2007; Coppa et al., 2013), milk samples derived from diets based only on conserved forages were attributed to the conserved forages group (**FH = 0**). This group was then divided into 3 subgroups, including milk samples derived from diets having at least 50% DM of corn silage (**CS >50-FH = 0**), hay (**H >50-FH = 0**), or grass silage (**GS >50-FH = 0**), whereas diets in which none of the conserved forages reached at least half of the DM proportion were assigned to the mixed feeding system (**MIX-FH = 0**).

(3) Level of Concentrate in the Cow Diet. Samples derived from diets containing concentrate (**C >0**) or not (**C = 0**) were assigned to groups. Within the C >0 group, samples were assigned to subgroups when the concentrate proportion in the diet was below 20% (**C <20**), between 20 and 30% (**C 20–30**), or above 30% of DM (**C >30**).

To better understand the role of fresh herbage or conserved forages in the authentication of the level of concentrate in the cow diet, the same class of concentrate supplementation level were applied within the FH = 0 group and within the FH > 50 group. Samples were thus assigned to **C <20-FH = 0**, **C 20–30-FH = 0**, or **C >30-FH = 0**, or to **C <20-FH >50**, **C 20–30-FH >50**, or **C >30-FH >50** for the FH = 0 or the FH > 50 groups, respectively.

(4) Altitude. Aiming to authenticate the altitude origin of milk from the FA composition, milk samples produced in upland or in lowland areas were assigned to groups. The general principles used in the different European states to define "upland areas" are based on considerable limitations in land-use possibilities responsible for an increase in the cost of farming practices, mainly due to the difficult climatic conditions, the presence of terrain unsuitable for the use of machinery, or a combination of these 2 factors (Santini et al., 2013). Considering these general principles, each European state, according to its latitude and climate, applies different threshold altitudes to define its upland area. In the present study, 3 categorization criteria for altitude group were used. (1) The local definition of a upland (considering both altitude and limitations in land use) of each state was used to assign the samples to the upland group: 700 m above sea level in France and Germany, 600 m above sea level in Italy, Slovenia, Slovakia, and Czech Republic. All the samples from Sweden and Norway were assigned to the upland group and all the samples from the Netherlands and Denmark were assigned to the lowland group. (2) As this approach reveals a combined effect of latitude, land-use limitation, and altitude, samples categorization according to altitude only was also performed. As a consequence, all the samples from Norway, Sweden, the Netherlands, and Denmark were assigned to the lowland group instead of the upland group, without any changes in the assignment of the samples from the other countries (data not shown). (3) Finally, samples from Norway, Sweden, the Netherlands, and Denmark were excluded for both lowland and upland groups (data not shown).

To test the ability of milk FA to differentiate milk originating from lowland or upland pasture, milk samples of the FH >50 group were divided according to altitude into lowland (**Low-FH >50**) and upland (**Up-FH >50**) groups. These groups reflect altitude-related differences in sward botanical composition and agronomic and grazing management. In the lowland group, temporary intensively managed grasslands were the dominant forage, whereas, in the upland group, agronomic practices were less intense and pastures were mainly permanent, extensively managed, and botanically diverse (Coppa et al., 2012b). The same approach

Table 1. Means and range of variation (in parentheses) of diet composition (% DM, unless otherwise noted) and altitude in the groups used to perform linear discriminant analyses for the authentication of cow diet and altitude of milk based on FA composition

Item[1]	n	Milk yield (kg/cow × day)	Fresh herbage	Grass silage	Hay	Corn silage	Total herbage-derived forages	Total conserved forages	Concentrates	Altitude (m above sea level)
FH >50	346	17.7 (6.1–31.5)	75 (50–100)	1 (0–23)	6 (0–35)	1 (0–35)	82 (52–100)	8 (0–45)	16 (0–48)	811 (1–2,100)
CS >50	32	23.6 (10.1–31)	3 (0–32)	7 (0–22)	7 (0–24)	60 (50–78)	17 (0–37)	74 (53–91)	23 (7–47)	392 (15–1,300)
H >50	105	16.9 (8.3–32)	2 (0–37)	1 (0–34)	70 (47–100)	0 (0–27)	73 (47–100)	72 (50–100)	25 (0–50)	882 (44–1,500)
GS >50	64	21.8 (13–29.6)	1 (0–29)	62 (50–78)	5 (0–33)	1 (0–17)	68 (50–90)	68 (50–92)	30 (0–50)	311 (50–1,000)
MIX	262	22.4 (6.5–40)	15 (0–50)	16 (0–50)	16 (0–49)	23 (0–49)	46 (9–94)	54 (0–92)	29 (6–63)	559 (15–1,700)
FH = 0	321	20.8 (6.5–40)	0 (0–3)	20 (0–77)	33 (0–100)	16 (0–78)	54 (0–100)	70 (37–100)	28 (0–63)	614 (15–1,500)
CS >50-FH = 0	26	23.5 (10.1–31.0)	0 (0–0)	8 (0–22)	8 (0–24)	60 (50–78)	15 (0–32)	76 (53–91)	24 (9–47)	415 (15–1,300)
H >50-FH = 0	100	17.1 (8.3–32.0)	0 (0–2)	2 (0–34)	71 (47–100)	0 (0–27)	72 (47–100)	73 (50–100)	26 (0–50)	881 (44–1,500)
GS >50-FH = 0	62	21.8 (13.0–29.6)	0 (0–0)	62 (50–77)	5 (0–33)	1 (0–17)	67 (50–90)	68 (50–92)	31 (5–50)	299 (50–1,000)
MIX-FH = 0	133	23.2 (6.5–40.0)	0 (0–3)	22 (0–50)	15 (0–49)	29 (0–49)	38 (9–80)	67 (37–92)	31 (8–63)	534 (50–1,300)
C = 0	34	16 (6.1–22)	86 (0–100)	2 (0–78)	10 (0–100)	1 (0–33)	98 (67–100)	13 (0–100)	0 (0–0)	927 (15–2,000)
C >0	775	19.8 (6.5–40)	32 (0–99)	11 (0–77)	21 (0–93)	11 (0–78)	64 (0–100)	43 (0–93)	25 (1–63)	675 (1–2,100)
C0–20	305	17.1 (7–31)	50 (0–99)	9 (0–74)	19 (0–93)	7 (0–78)	78 (5–100)	36 (0–93)	14 (1–20)	752 (1–2,100)
C20–30	244	20.4 (8.7–37.7)	21 (0–80)	12 (0–77)	23 (0–80)	16 (0–70)	57 (7–80)	52 (0–80)	26 (20–30)	633 (15–1,700)
C >30	226	22.5 (6.5–40)	16 (0–70)	13 (0–69)	21 (0–69)	10 (0–60)	51 (0–70)	45 (0–70)	39 (30–63)	606 (50–1,650)
C0–20-FH = 0	64	16.9 (8.3–25)	0 (0–2)	24 (0–74)	43 (0–93)	15 (0–78)	67 (5–93)	82 (62–93)	15 (5–20)	594 (15–1,500)
C20–30-FH = 0	130	20.1 (10.3–31)	0 (0–1)	19 (0–77)	33 (0–80)	20 (0–70)	52 (7–80)	72 (51–80)	26 (20–30)	621 (50–1,500)
C >30-FH = 0	127	23 (6.5–40)	0 (0–3)	20 (0–69)	27 (0–69)	14 (0–60)	47 (0–70)	61 (37–70)	39 (30–63)	618 (50–1,500)
C0–20-FH >50	164	17.1 (7–31)	76 (50–99)	1 (0–23)	8 (0–35)	2 (0–35)	85 (55–100)	10 (0–37)	13 (1–20)	881 (1–2,100)
C20–30-FH >50	56	17.9 (8.7–31.5)	67 (50–80)	1 (0–11)	7 (0–24)	1 (0–15)	74 (60–80)	9 (0–28)	25 (20–30)	763 (50–1,700)
C >30-FH >50	62	21.6 (13.8–27.6)	60 (50–70)	0 (0–0)	2 (0–16)	0 (0–0)	62 (52–70)	2 (0–16)	38 (30–48)	363 (50–1,650)
Low	307	21.2 (7.1–40)	24 (0–100)	12 (0–78)	20 (0–93)	20 (0–78)	57 (0–100)	52 (0–93)	22 (0–63)	396 (1–690)
Up	502	18.5 (6.1–39)	43 (0–100)	9 (0–77)	21 (0–100)	3 (0–61)	73 (10–100)	33 (0–100)	24 (0–61)	911 (15–2,100)
Low-FH >50	79	18.7 (7.1–31.5)	72 (50–100)	1 (0–23)	10 (0–32)	3 (0–35)	83 (55–100)	14 (0–45)	12 (0–37)	405 (1–670)
Up-FH >50	267	17.4 (6.1–31)	76 (50–100)	0 (0–20)	5 (0–35)	1 (0–26)	82 (52–100)	6 (0–35)	17 (0–48)	958 (50–2,100)
Low-FH = 0	147	22.6 (10.1–40)	0 (0–3)	19 (0–74)	27 (0–93)	26 (0–78)	46 (0–93)	71 (37–93)	26 (0–63)	404 (15–690)
Up-FH = 0	174	19.4 (6.5–35)	0 (0–2)	22 (0–77)	40 (0–100)	6 (0–61)	63 (10–100)	68 (39–100)	31 (0–61)	834 (50–1,500)

[1]C = concentrate; CS = corn silage; FH = fresh herbage; GS = grass silage; H = hay; Low = lowland; MIX = mixed diets; Up = upland; numbers indicate the proportion of different feeding in cow diet (on DM basis) used as threshold for the assignment of samples to the groups.

Table 2. Means and range (in parentheses) of milk FA profiles in the calibration and validation sets

	Calibration set		Validation set	
FA (g/100 g of FA)	n	Mean (range)	n	Mean (range)
C12:0	990	3.53 (2.05–5.97)	247	3.49 (2.09–5.81)
iso C14:0	974	0.15 (0.05–0.30)	242	0.15 (0.06–0.30)
C14:0	995	11.81 (8.31–14.94)	248	11.69 (8.22–14.84)
iso C15:0	959	0.32 (0.15–0.52)	236	0.31 (0.16–0.52)
anteiso C15:0	959	0.58 (0.32–0.99)	236	0.58 (0.34–1.01)
C15:0	993	1.26 (0.79–1.74)	246	1.24 (0.77–1.77)
iso C16:0	912	0.33 (0.15–0.55)	222	0.33 (0.16–0.49)
C16:0	987	29.12 (21.57–39.13)	248	28.88 (22.22–38.12)
iso C17:0 + C16:1 *trans*-9	926	0.47 (0.22–0.80)	231	0.47 (0.23–0.80)
C16:1 *cis*-9 + *anteiso* C17:0	942	1.87 (1.13–2.86)	230	1.88 (1.23–2.47)
C17:0	994	0.68 (0.39–1.01)	247	0.68 (0.40–0.94)
iso C18:0	865	0.054 (0.026–0.096)	210	0.054 (0.025–0.091)
C17:1 *cis*-9	817	0.24 (0.12–0.48)	207	0.24 (0.13–0.46)
C18:0	985	9.48 (5.38–13.29)	244	9.61 (5.95–13.02)
C18:1 *trans*-10	588	0.24 (0.03–0.54)	145	0.25 (0.10–0.50)
C18:1 *trans*-11	592	1.7 (0.45–5.81)	146	1.73 (0.49–3.95)
C18:1 *trans*-10 + *trans*-11	997	2.07 (0.51–6.22)	247	2.13 (0.63–5.65)
C18:1 *cis*-9 + *trans*-13	993	19.28 (12.18–28.95)	248	19.66 (13.18–27.98)
C18:2 *cis*-9,*trans*-13	708	0.17 (0.01–0.41)	172	0.18 (0.01–0.43)
C18:2 *trans*-11,*cis*-15	710	0.20 (0.01–0.86)	172	0.22 (0.02–0.91)
C18:2n-6	902	1.43 (0.51–2.40)	215	1.42 (0.71–2.40)
C18:3n-3	990	0.67 (0.22–1.57)	247	0.66 (0.22–1.59)
CLA *cis*-9,*trans*-11	996	0.88 (0.21–2.8)	248	0.91 (0.23–2.58)
C20:4n-6	875	0.085 (0.030–0.170)	209	0.083 (0.029–0.178)
C20:5n-3	872	0.06 0(0.010–0.120)	210	0.060 (0.022–0.120)
iso C15:0/*iso* C14:0	957	2.14 (1.13–3.93)	236	2.13 (1.20–3.77)
C16:1 *cis*-9 + *anteiso* C17:0/*iso* C16:0	895	5.91 (2.93–13.80)	216	6.01 (3.60–14.76)
C18:2 *trans*-11,*cis*-15/C18:1 *trans*-11	710	0.084 (0.004–0.242)	171	0.088 (0.011–0.236)

was adopted in dividing the FH = 0 into a lowland (**Low-FH = 0**) and an upland (**Up-FH = 0**) group, aiming to test the ability of milk FA to authenticate milk originating from lowland or upland areas during the season in which fresh herbage is not available.

Statistics

Statistical models to predict diet composition using the milk FA profile were performed according to the procedure described by Coppa et al. (2013). Briefly, the samples were divided randomly into 2 sets: a calibration set (80% of the samples corresponding to 999 FA profiles) used to calibrate the models and a validation set (249 randomly selected FA profiles) used to perform external validation of the models. To develop a prediction model of each feedstuff, a general linear model was applied using experiment as fixed factor and FA concentrations as covariates. A stepwise approach was used to identify significant covariates. The mean, minimum, and maximum concentrations for each FA used to develop the prediction equations are reported in Table 2 for both calibration and validation sets. The significance level was set to $P < 0.05$. The linear, quadratic, and cubic effects of all the covariates were tested. Root mean square error (**RMSE**) and R^2 were used to describe model fitting for both calibration and external validation. The Fisher's F value of each variable included in a model was used as an indicator of the relative weight of the variable in determining the model itself. Prediction models were performed with Minitab 14.1 (Minitab Inc., State College, PA).

To authenticate feeding system and altitude groups, linear discriminant analyses were performed using FA found significant in the prediction models of diet composition and altitude as variables. The resulting discrimination functions were validated by the "leave-one-out" method (full cross-validation). The SPSS for Windows software package (version 17.0; SPSS Inc., Chicago, IL) was used for the linear discriminant analyses.

RESULTS AND DISCUSSION

The variability of the data set for both production conditions and FA composition was very large. Its representativeness of farming systems located from lowland to upland areas in Europe and of almost all the farming systems found in Europe is high and has been already discussed by Coppa et al. (2013).

Prediction of Diet Composition and Altitude with Models Based on Milk FA Composition

The models for each variable describing diet composition and altitude are reported in Tables 3 and 4. Mod-

Table 3. Precision of the prediction models of cow diet composition (% of diet DM) and altitude (meters above sea level) based on milk FA composition

	Calibration[1]			Validation[1]		
Production condition	n	RMSE	R^2	n	RMSE	R^2
Fresh herbage	693	15.6	0.81	162	15.1	0.79
Corn silage	678	9.1	0.66	156	7.6	0.66
Hay	683	11.2	0.75	164	8.8	0.74
Grass silage	659	10.6	0.62	156	8.4	0.62
Concentrate	883	8.1	0.51	159	6.3	0.50
Herbage-derived forage	828	10.7	0.73	198	9.8	0.73
Conserved forage	943	15.2	0.75	196	13.6	0.74
Altitude	842	233	0.62	198	123	0.60

[1]n = number of samples; RMSE = root mean square error.

els were significant (data not shown) for all the studied variables, and the R^2 found in calibration models were confirmed by the external validation. The proportion of different feedstuffs in cow diets and the altitude were related linearly to several milk FA concentrations, but quadratic or cubic relations were also found for each model. Models for each variable describing diet composition and altitude are discussed in the following paragraphs.

Fresh Herbage. The proportion of fresh herbage in cow diet was reliably predicted by the model (R^2 = 0.81; RMSE = 15.6). The FA explaining most of the model variability were C18:2 *trans*-11,*cis*-15, and *iso* C17:0 + C16:1 *trans*-9 (Fisher's $F > 84$), both linearly and positively related to the proportion of fresh herbage in the diet. The C18:2 *trans*-11,*cis*-15 is an intermediate of ruminal biohydrogenation of dietary C18:3n-3, which is the main FA in herbage (Chilliard et al., 2007), and its increase in milk with an increasing proportion of fresh herbage in cow diet is well known (Couvreur et al., 2006; Chilliard et al., 2007; Ferlay et al., 2008). Vlaeminck et al. (2006) showed a linear increase of *iso* C17:0 with an increase in the major biohydrogenation intermediates in milk, such as C18:2 *trans*-11,*cis*-15. In our model, as expected, the proportion of fresh herbage in the diet also decreased linearly with C16:0 (Couvreur et al., 2006; Chilliard et al., 2007). It also increased linearly with C17:1 *cis*-9 and decreased cubically with C17:0 concentration in milk (Fisher's $F > 31$). These opposite effects resulted in a global positive effect of C17:0 + C17:1 *cis*-9. The concentrations of these 2 FA are strongly correlated, as C17:1 *cis*-9 originates from Δ9-desaturation of C17:0 in the mammary gland (Vlaeminck et al., 2006). An increase in fresh herbage proportion in cow diet was already associated with a linear increase in C17:0 in milk by Couvreur et al. (2006). Surprisingly C18:1 *trans*-11 + *trans*-10 had only a slight weight in determining fresh herbage prediction model (Fisher's $F < 15$), whereas CLA *cis*-9,*trans*-11 was not significant. The weight of these FA in the models was similar when using C18:1 *trans*-11 and C18:1 *trans*-10 concentration separately on a subsample set (R^2 calibration = 0.82; R^2 validation = 0.79; data not shown). However, we have to consider that the botanical composition and phenological stage of fresh forage are important drivers of the milk content in these FA (Dewhurst et al., 2006; Ferlay et al., 2006) but were not available in the current study. Recording on commercial farms, Coppa et al. (2012b) suggested that with more than 70% of fresh herbage in the diet, the differences in bulk milk FA composition depend mainly on herbage botanical composition and phenological stage and grazing management. The absence of data related to the fresh herbage quality could also explain the quite high RMSE of the model even though the proportion of fresh herbage in the diet was well fitted.

Corn Silage. The model for the proportion of corn silage had a poorer fit (R^2 = 0.66; RMSE = 9.1) than the fresh herbage model. Most of the model variability was explained by the C16:1 *cis*-9 + *anteiso* C17.0-to-*iso* C16:0 ratio (Fisher's $F > 73$), whose linear increase was associated with an increased proportion of corn silage in the diet. High values of this FA ratio were associated by Engel et al. (2007) with milk derived from diets with a proportion of corn silage greater than 30% of DM, together with a lower C18:2 *trans*-11,*cis*-15-to-C18:1 *trans*-10 + *trans*-11 ratio. A decrease in the C18:2 *trans*-11,*cis*-15-to-C18:1 *trans*-10 + *trans*-11 ratio in our model was indeed linearly related to the increase of corn silage proportion in the diet, but its weight was minimal (Fisher's $F = 8.7$). As expected, an increase in the proportion of corn silage fed was also related to a decrease in C18:3n-3 and in C20:5n-3 concentrations in milk (Fisher's $F > 23$), as corn silage is low in C18:3n-3 and other n-3 PUFA and rich in C18:2n-6 (Dewhurst et al., 2006; Chilliard et al., 2007).

Hay. Most of the variability of the prediction model for the proportion of hay in the diet ($R^2 > 0.74$; RMSE = 11.2) was explained by milk *iso* C14:0 and C18:3n-3 concentrations and the C18:2 *trans*-11,*cis*-15-to-C18:1

Table 4. Equations of the prediction models of cow diet composition (% DM) and altitude based on milk FA composition (g/100 g of FA)

Production condition	Parameters of the model[1]
Fresh herbage	177.4 ± 39.83 (*F* experiment *7.9*) + 122.2 ± 11.86 × *iso* C17:0 + C16:1 *trans*-9 (*F 106.1*) + 78.65 ± 8.554 × C18:2 *trans*-11,*cis*-15 (*F 84.5*) + 363.7 ± 56.13 × C17:1 *cis*-9 (*F 42.0*) − 65.0 ± 10.18 × C17:0^3 (*F 40.8*) − 10.71 ± 1.923 × C16:0 (*F 31.0*) − 12.97 ± 2.596 × C18:2n-6 (*F 25.0*) − 2,361 ± 513.0 × C18:2 *trans*-11,*cis*-15^3 (*F 21.2*) − 1,145 ± 279.6 × C17:1 *cis*-9^3 (*F 16.8*) + 0.0028 ± 0.00069 × C16:0^3 (*F 16.0*) + 665.7 ± 170.20 × C18:2 *cis*-9,*trans*-13^2 (*F 15.3*) − 0.1696 ± 0.0444 × C18:1 *trans*-10 + *trans*-11^3 (*F 14.6*) − 94.8 ± 38.72 × *iso* C16:0^3 (*F 6.0*)
Corn silage	−383.0 ± 89.58 (*F* experiment *9.0*) + 3.363 ± 0.3920 × C16:1 *cis*-9 + *anteiso* C17:0/*iso* C16:0 (*F 73.5*) − 141.1 ± 25.28 × C18:3n-3 (*F 31.1*) − 235.0 ± 46.24 × C20:5n-3 (*F 25.8*) + 158.0 ± 32.51 × C18:3n-3^2 (*F 23.6*) + 164.1 ± 36.01 × C20:4n-6 (*F 20.8*) + 893.5 ± 219.70 × C15:0 (*F 16.5*) − 50.92 ± 13.390 × C18:3n-3^3 (*F 14.5*) − 644.5 ± 177.00 × C15:0^2 (*F 13.3*) − 2.581 ± 0.7638 × C18:2n-6^3 (*F 11.4*) + 153.6 ± 46.97 × C15:0^3 (*F 10.7*) − 56.01 ± 18.970 × C18:2 *trans*-11,*cis*-15/C18:1 *trans*-10 + *trans*-11 (*F 8.7*) − 0.00048 ± 0.00017 × C18:1 *cis*-9 + *trans*-13^3 (*F 7.7*) + 165.4 ± 72.03 × C18:2 *cis*-9,*trans*-13^3 (*F 5.3*) + 11.40 ± 6.111 × C18:2n-6 (*F 3.5*)
Hay	86.52 ± 47.880 (*F* experiment *4.8*) + 641.9 ± 56.77 × *iso* C14:0^2 (*F 127.9*) − 248.4 ± 22.62 × C18:2 *trans*-11,*cis*-15/C18:1 *trans*-10 + *trans*-11 (*F 120.6*) + 129.5 ± 12.23 × C18:3n-3 (*F 112.1*) − 64.25 ± 6.628 × C18:3n-3^2 (*F 94.0*) + 2.325 ± 0.2830 × C16:0 (*F 67.7*) + 280.0 ± 41.13 × C17:0^2 (*F 46.3*) − 33.35 ± 5.611 × C15:0 (*F 35.3*) − 344.1 ± 59.45 × C17:0 (*F 33.5*) + 50.85 ± 10.920 × C18:2 *cis*-9,*trans*-13 (*F 21.7*) − 4.24 ± 0.919 × C18:1 *trans*-10 + *trans*-11 (*F 21.3*) + 0.804 ± 0.3040×C14:0^2 (*F 7.0*) + 216.1 ± 89.38×C20:4n-6 (*F 5.8*) − 14.94 ± 7.277 × C14:0 (*F 4.2*) + 102.3 ± 58.05 × C20:5n-3 (*F 3.1*) − 4,804 ± 2,904.0 × C20:4n-6^3 (*F 2.7*)
Grass silage	265.4 ± 40.36 (*F* experiment *9.3*) − 285.4 ± 21.50 × *iso* C17:0 + C16:1 *trans*-9 (*F 176.2*) − 1.423 ± 0.1525 × C12:0^2 (*F 87.0*) + 220.0 ± 24.19 × *iso* C17:0 + C16:1 *trans*-9^3 (*F 82.7*) + 15.77 ± 1.882 × C15:0^2 (*F 70.3*) − 81.57 ± 10.050 × C18:2 *cis*-9,*trans*-13 (*F 65.9*) − 208.4 ± 28.42 × *iso* C14:0 (*F 53.8*) −16.43 ± 3.063 × C16:1 *cis*-9 + *anteiso* C17:0/*iso* C16:0 (*F 28.8*) + 76.14 ± 18.570 × C18:2 *trans*-11,*cis*-15/C18:1 *trans*-10 + *trans*-11 (*F 16.8*) − 405.7 ± 105.60 × *iso* C16:0 (*F 14.8*) + 3.26 ± 0.910 × C18:2n-6^3 (*F 12.8*) + 515.8 ± 151.80 × iso C16:0^3 (*F 11.6*) − 23.89 ± 7.106 × C18:2n-6 (*F 11.3*) + 3.80 ± 1.252 × C16:0 (*F 9.2*) − 0.00137 ± 0.00046 × C16:0^3 (*F 8.9*) + 27.64 ± 9.322 × C16:1 *cis*-9 + *anteiso* C17:0 (*F 8.8*) −87.51 ± 39.170 × C20:4n-6 (*F 5.0*)
Concentrate	131.3 ± 10.78 (*F* experiment *11.5*) − 235.6 ± 37.22 × *iso* C14:0 (*F 40.1*) − 2.57 ± 0.452 × C16:1 *cis*-9 + *anteiso* C17:0/*iso* C16:0 (*F 32.5*) + 1.04 ± 0.188 × C18:2n-6^3 (*F 30.4*) − 111.6 ± 23.94 × C17:0 (*F 21.7*) + 1,690 ± 364.0 × *iso* C14:0^3 (*F 21.6*) − 3.26 ± 0.751 × C18:1 *trans*-10 + *trans*-11 (*F 18.8*) + 50.97 ± 14.380 × C17:0^3 (*F 12.6*) − 21.6 ± 6.41 × *iso* C17:0 + C16:1 *trans*-9^2 (*F 11.4*) − 13.5 ± 4.20 × C18:3n-3^2 (*F 10.4*) + 19.51 ± 6.734 × C18:3n-3 (*F 8.4*) −190 ± 66.9 × C18:2 *cis*-9,*trans*-13^3 (*F 8.1*)
Herbage-derived forages	−0.77 ± 18.990 (*F* experiment *5.6*) + 39.01 ± 5.627 × *iso* C17:0 + C16:1 *trans*-9 (*F 48.1*) + 370.2 ± 62.67 × *iso* C14:0 (*F 34.9*) + 160.3 ± 28.15 × C18:3n-3 (*F 32.4*) − 169.6 ± 34.94 × C18:3n-3^2 (*F 23.6*) − 860.8 ± 184.40 × *iso* C14:0^2 (*F 21.8*) + 61.32 ± 13.830 × C18:3n-3^3 (*F 19.7*) − 124.0 ± 31.15 × C20:4n-6 (*F 15.8*) − 8.13 ± 2.297 × C18:2n-6 (*F 12.5*) + 454.5 ± 152.50 × C20:5n-3 (*F 8.9*) + 0.114 ± 0.0490 × C18:1 *cis*-9 + *trans*-13^2 (*F 5.4*) − 2,165 ± 1,072.0 × C20:5n3^2 (*F 4.1*) − 3.70 ± 1.879 × C18:1 *cis*-9 + *trans*-13 (*F 3.9*)
Conserved forages	1,593 ± 288.3 (*F* experiment *2.9*) − 18.08 ± 1.880 × C18:1 *trans*-10 + *trans*-11 (*F 92.0*) − 116.7 ± 13.69 × *anteiso* C15:0 (*F 72.6*) − 0.067 ± 0.0110 × C16:0^3 (*F 40.7*) + 5.974 ± 0.9590 × C16:0^2 (*F 38.8*) − 173.7 ± 28.96 × C16:0 (*F 36.0*) + 0.347 ± 0.0579 × C18:1 *trans*-10 + *trans*-11^3 (*F 35.9*) + 31.19 ± 6.130 × C15:0 (*F 25.9*) + 116.4 ± 28.09 × iso C16:0^2 (*F 17.2*) + 1.091 ± 0.3130 × C18:2n-6^3 (*F 12.1*) + 8,928 ± 2967.0 × *iso* C18:0^3 (*F 9.1*) + 15.33 ± 5.110 × C14:0 (*F 9.0*) + 272.9 ± 105.40 × *iso* C14:0^2 (*F 6.7*) − 0.0293 ± 0.0114 × C14:0^3 (*F 6.6*)
Altitude (m above sea level)	−1,404 ± 315.3 (*F* experiment *37.2*) + 397.5 ± 51.28 × C18:2n-6 (*F 60.1*) + 6,426 ± 1,246.0 × *iso* C14:0 (*F 26.6*) − 2,777 ± 637.8 × C17:0^3 (*F 19.0*) − 3,747 ± 860.9 × *iso* C15:0^2 (*F 18.9*) − 2,870 ± 689.1 × C20:4n-6 (*F 17.4*) + 2,817 ± 712.7 × C17:0^2 (*F 15.6*) + 43.0 ± 12.31 × CLA *cis*-9,*trans*-11^2 (*F 12.2*) + 220.0 ± 90.65 × *iso* C15:0/*iso* C14:0 (*F 5.9*) + 110.0 ± 48.75 × C16:1 *cis*-9 + *anteiso* C17:0 (*F 5.1*) − 8.82 ± 4.308 × C18:1 *cis*-9 + *trans*-13 (*F 4.2*) + 408.3 ± 211.20 × *anteiso* C15:0 (*F 3.7*) + 108.5 ± 57.93 × C18:3n-3 (*F 3.5*)

[1]Each equation is presented in the following format: intercept of the model (Fisher's *F* of the effect experiment) + coefficient ± SE × FA (Fisher's *F*).

trans-10 + *trans*-11 ratio (Fisher's $F > 112$). The positive quadratic relation between milk *iso* C14:0 and the proportion of hay can be easily explained by the microbial origin of this FA, which derives from ruminal cellulolytic bacteria, increasing with an increasing proportion of forage in the diet (Vlaeminck et al., 2006). Considering the positive linear and negative quadratic relations between the proportion of hay and C18:3n-3 concentration in milk, the resulting global relation is positive when C18:3n-3 concentration is lower than 1 g/100 g of FA and negative for higher concentrations. This FA concentration in milk depends on its dietary supply and on the extent of its biohydrogenation in the rumen (Chilliard et al. 2007). The positive relation between the proportion of hay and C18:3n-3 concentration in milk is in agreement with the high concentration of C18:3n-3 in milk from hay-based diets (Ferlay et al., 2006). This high concentration was explained by a high transfer efficiency of this FA from hay to milk due to a putative low ruminal biohydrogenation (Chilliard et al., 2007). For the highest C18:3n-3 concentration in milk, the negative relation between this FA and the proportion of hay indicates that an additional increase of C18:3n-3 in milk reveals a switch from hay to other

feedstuffs, leading to an even higher C18:3n-3 concentration in milk, namely fresh herbage in this case. Finally, the negative linear relation we found between the C18:2 *trans*-11,*cis*-15-to-C18:1 *trans*-10 + *trans*-11 ratio and the proportion of hay agrees with the low ruminal biohydrogenation of C18:3n-3 suggested by Chilliard et al. (2007) to explain the high C18:3n-3 concentration of hay-derived milk. Indeed, C18:2 *trans*-11,*cis*-15, a specific intermediate of C18:3n-3 ruminal biohydrogenation (Chilliard et al., 2007), decreases in milk when the proportion of hay in diet increases. Similar to fresh herbage, the absence of data related to hay quality (phenology, barn drying, weather conditions, and mechanical treatment during harvesting and drying) could likely explain the relatively high RMSE of the model for hay and for all the models including herbage-derived feedstuffs.

Grass Silage. The model fit for the proportion of grass silage was similar to that for corn silage (R^2 = 0.62; RMSE = 10.6). The proportion of grass silage in the diet decreased linearly with increasing milk *iso* C17:0 + C16:1 *trans*-9 concentration (Fisher's F = 176.2). This trend is in agreement with Vlaeminck et al. (2006), who showed an increase in this FA when grass silage was replaced by corn silage in cow diet. This strong negative linear relation overcomes the positive cubic relation reported in the model. The C12:0 and C15:0 were negatively and positively related to grass silage, respectively (Fisher's $F > 70$), in agreement with the lower C12:0 and higher C15:0 concentration in milk found with grass silage-based diets in comparison to corn silage-based diets (Vlaeminck et al., 2006; Hurtaud et al., 2009; Ferlay et al., 2013). An increase in C18:2 *cis*-9,*trans*-13 (Fisher's $F = 65.9$) was linearly related to a decrease in grass silage concentration. This FA could contribute to explain the model variability related to those diets in which grass silage was substituted with other feedstuffs rather than corn silage, such as fresh herbage, as occurs when cows are turned out at pasture. Indeed a higher concentration in C18:2 *cis*-9,*trans*-13 in milk could be linked to the higher concentration of the intermediates of ruminal biohydrogenation of ingested C18:3n-3 when grass silage is substituted by fresh herbage (Ferlay et al., 2008; Shingfield et al., 2013).

Concentrates. The lowest model reliability was found for the prediction of the proportion of concentrates in the diet ($R^2 < 0.51$; RMSE = 8.1). The FA explaining most of the model variability were *iso* C14:0, C18:2n-6 ($F > 30$), and the C16:1 *cis*-9 + *anteiso* C17:0-to-*iso* C16:0 ratio. The concentrate proportion decreased when *iso* C14:0 increased linearly and increased when increasing quadratically the C18:2n-6 concentration in milk (Fisher's $F > 30$). These FA are known to be related to dietary starch content and to forage concentrate ratios (Vlaeminck et al., 2006; Chilliard et al., 2007; Sterk et al., 2011). A linear decrease in C16:1 *cis*-9 + *anteiso* C17:0-to-*iso* C16:0 ratio (Fisher's $F = 32.5$) was associated with an increase in concentrate proportion in the diet in our model. This suggests that this FA ratio may be related to dietary starch content and thus, indirectly, to the proportion of corn silage as already shown by Engel et al. (2007). The model included also the C18:1 *trans*-10 + *trans*-11, but this FA had a low weight in the model determination (Fisher's $F = 18.8$). When calibrating the model using C18:1 *trans*-11 and C18:1 *trans*-10 as separate isomers instead of their sum, the model fit was slightly improved ($R^2 < 0.65$; RMSE = 7.4; data not shown), likely because of the well-known switch from the C18:1 *trans*-11 to C18:1 *trans*-10 isomer in the case of diets rich in concentrates and, consequently, in starch (Chilliard et al., 2007). The main FA driving the model for concentrate prediction in the cow diet seemed to be indicators of starch content in cow diet. However, several by-products, poor in starch, are currently used as concentrate in cow diet (i.e., soybean meal, rapeseed meal, lipids, and so on) with different and contrasting effects on milk FA composition (Borreani et al., 2013), concurring to explain the relatively low fit of the model.

Herbage-Derived and Conserved Forages. The variability of the model for the prediction of total herbage-derived forages (R^2 = 0.73) was mainly explained by *iso* C17:0 + C16:1 *trans*-9, *iso* C14:0, and C18:3n-3 (Fisher's $F > 30$), with similar trends to those observed for the fresh herbage and hay. Most of the variability of the total conserved forages prediction model ($R^2 >$ 0.74) was explained by C18:1 *trans*-10 + *trans*-11, *anteiso* C15:0, and C16:0 (Fisher's $F > 35$). Considering the positive negative linear and positive cubic relations between the proportion of conserved forages and C18:1 *trans*-10 + *trans*-11 concentration in milk, the resulting global relation is negative, except for the highest C18:1 *trans*-10 + *trans*-11 concentrations (>5 g/100 g of FA) for which the relation is positive. Due to the switch from conserved forages to fresh herbage while conserved forage proportion in the diet decreases, this trend is in agreement with the increased proportion of fresh herbage in the diet (Couvreur et al., 2006; Chilliard et al., 2007). The highest C18:1 *trans*-10 + *trans*-11 proportion reveals a switch from conserved forages to concentrates that could explain the positive relation we found when the C18:1 *trans*-10 + *trans*-11 proportion is greater than 5 g/100 g of FA (Chilliard et al., 2007, Sterk et al., 2011). The global relation between total conserved forages and C16:0 concentration is positive when C16:0 range from 25 to 35 g/100 g of FA and negative for lower and higher values. The positive link

reveals a switch from conserved forages to fresh herbage. Out of this range, the effect depends on which concentrate or feeds are displaced in the diet (Chilliard et al., 2007; Ferlay et al., 2008; Sterk et al., 2011).

Altitude. The model fit for altitude ($R^2 < 0.62$) was similar to those of corn silage and grass silage ($R^2 < 0.66$). Altitude was positively linearly related to *iso* C14:0 and C18:2n-6 concentrations (Fisher's $F > 26$). A high concentration of *iso* C14:0 in milk is consistent with a high proportion of fresh or conserved herbage (mainly hay), rich in fiber, in upland dairy farming systems (Coppa et al., 2012a; Ferlay et al., 2008). An increase in C18:2n-6 concentration of milk from cows grazing on upland pastures when compared with conserved forage-based diets has also been previously observed (Leiber et al., 2005; Coppa et al., 2012a). Indeed, an increase in C18:2n-6 and C18:3n-3 (following the same trend of C18:2n-6 in the model, but with a lower Fisher's *F*) concentrations in the milk of grazing cows with altitude have been explained by Leiber et al. (2005) by a possible inhibitory effect on ruminal PUFA biohydrogenation due to the presence in plant secondary metabolites of dicotyledonous species, which are abundant in upland pasture. The relatively high RMSE was an expected result and can be easily explained by the variation in milk FA composition at similar altitude related to the variations in farming practices and herbage characteristics (Coppa et al., 2012b).

Authentication of Cow-Feeding System and Altitude Origin using Linear Discriminant Analyses Based on Milk FA Composition

Feeding System. When discriminating among all feeding systems, only 75.9% of samples were correctly classified (Table 5), although this was significantly improved when removing the mixed diets from the data set (90.1% of samples correctly classified), as already observed by Coppa et al. (2012b). These very high discriminating performances were quite unexpected, especially considering the heterogeneity of the present data set (Coppa et al., 2013). The results were improved further still when discriminating between pairs of feeding systems (Table 5), reaching performance among the highest found in literature for cow feeding authentication using stable isotopes or IR technologies (Renou et al., 2004; Coppa et al., 2012b, Valenti et al., 2013). When discriminating between fresh herbage- and corn silage-feeding systems (96.8% of samples were correctly classified), the FA contributing the most to the discriminant function were C16:0 and the C16:1 *cis*-9 + *anteiso* C17:0-to-*iso* C16:0 ratio ($r > 0.39$), positively related to the corn silage-feeding system, and C18:3n-3, *iso* C17:0 + C16:1 *trans*-9, C18:2 *trans*-11,*cis*-15-to-C18:1 *trans*-10 + *trans*-11 ratio, *anteiso* C15:0, and *iso* C15:0 ($r > 0.35$), positively related to fresh herbage-feeding system. Hurtaud et al. (2014) also found an important contribution of odd- and branched-chain FA in the discrimination between milk derived from feeding systems based on fresh herbage and milk derived from corn silage-based diets. The contribution of the C16:1 *cis*-9 + *anteiso* C17:0-to-*iso* C16:0 ratio and of the C18:2 *trans*-11,*cis*-15-to-C18:1 *trans*-10 + *trans*-11 ratio, found by Engel et al. (2007) to authenticate corn silage- and fresh herbage-feeding systems, is confirmed in our study. However, the 2 FA ratios were not powerful enough to be used alone to achieve high discrimination, perhaps because of the extensive data set in our study compared with those of Engel et al. (2007), who collected a small number of milk samples from a restricted geographical area.

The discrimination between fresh herbage- and hay-feeding systems was also very good (96.7% of samples correctly classified) and similar to those obtained by Coppa et al. (2011) using volatile compounds. The main FA contributing to the discriminant function were C16:0 and C14:0 (r: >0.37), associated with a hay-feeding system, and C18:1 *trans*-10 + *trans*-11, C18:0, C18:2 *trans*-11,*cis*-15, C18:1 *cis*-9 + *trans*-13, and CLA *cis*-9,*trans*-11 (r: >0.35), related to a fresh herbage-feeding system.

The discriminating performance between fresh herbage- and grass silage-feeding systems (93.7% of sample correctly classified) was similar to those found by Coppa et al. (2012b) with IR spectra. The FA having the highest weight in the determination of the discriminant function were C16:0 (r: 0.52), related to ensiled herbage-feeding systems, and *iso* C17:0 + C16:1 *trans*-9, *anteiso* C15:0, *iso* C15:0, CLA *cis*-9,*trans*-11, C18:1 *trans*-10 + *trans*-11, and C18:3n-3 (r: >0.41), associated with fresh herbage-feeding systems.

Conserved Forage-Based Feeding System. When considering only milk samples deriving from conserved forage-based diets, the discriminating performance among feeding systems was improved (84.7% of samples correctly classified) compared with those obtained on the whole data set (Table 6). However, the performances were similar when samples derived from mixed diets were excluded. We also obtained very good discrimination between pairs of conserved forage-feeding systems (Table 6). When discriminating between hay- and grass silage-feeding systems (96.9% of samples correctly classified), the FA having the greatest influence were C18:0 (r: 0.38), associated with ensiled herbage-feeding systems, and *iso* C14:0, *iso* C15:0, *anteiso* C15:0, and C18:3n-3 (r: >0.35), associated with hay-feeding systems. The relevance of our results is highlighted by the smaller differences in milk FA composition between

Table 5. Classification results of the discriminant analyses based on milk FA composition to authenticate cow-feeding system

Discriminant analysis	Group[1]	Total no. of samples	Correct classification in cross-validation	
			n	%
All feeding systems, including mixed diets	FH >50	346	292	84.4
	CS >50	32	25	78.1
	H >50	105	89	84.8
	GS >50	64	57	89.1
	MIX	262	151	57.6
	All	809	614	75.9
All feeding systems, excluding mixed diets	FH >50	346	315	91.0
	CS >50	32	25	78.1
	H >50	105	94	89.5
	GS >50	64	59	92.2
	All	547	493	90.1
Fresh herbage vs. conserved forage	FH >50	346	325	93.9
	FH = 0	321	306	95.3
	All	667	631	94.6
Fresh herbage vs. corn silage	FH >50	346	341	98.6
	CS >50	32	25	78.1
	All	378	366	96.8
Fresh herbage vs. hay	FH >50	346	337	97.4
	H >50	105	99	94.3
	All	451	436	96.7
Fresh herbage vs. grass silage	FH >50	346	323	93.4
	GS >50	64	61	95.3
	All	410	384	93.7

[1]CS = corn silage; FH = fresh herbage; GS = grass silage; H = hay; MIX = mixed diets; numbers indicate the proportion of different feeds in the diet (on DM basis) used as threshold for the assignment of samples to the groups.

hay- and grass silage-derived milk compared with those between hay- and either fresh herbage- or corn silage-derived milk (Ferlay et al., 2006). Indeed, the discrimination between hay- and grass silage-feeding systems has rarely been reported in the literature or tested in controlled conditions (Renou et al., 2004). Recording on commercial farms, their discrimination was found imprecise by Coppa et al. (2012b) by using IR. The best discriminating performance was observed between hay- and corn silage-feeding systems (98.4% of samples correctly classified). This result is quite surprising when considering the poorer discrimination obtained with other techniques (Renou et al., 2004; Coppa et al., 2012b; Valenti et al., 2013). However, marked differences in milk FA composition were observed when comparing hay- and corn silage-based diets (Ferlay et al., 2006, 2008). The FA contributing the most to discrimination were the C16:1 *cis*-9 + *anteiso* 17-to-*iso* C16:0 ratio (r: 0.34), related to corn silage systems, and *iso* C14:0, C18:3n-3, and *iso* C15:0 (r: >0.40). The C16:1 *cis*-9 + *anteiso* 17-to-*iso* C16:0 ratio was also related to corn silage systems (r: 0.34), discriminating it from grass silage systems (96.6% of samples correctly classified) and confirming the role of this FA ratio to identify milk from corn silage-feeding systems (Engel et al., 2007).

Concentrate Supplementation. The milk FA concentrations were an useful tool to identify the presence of concentrates in cow diets, with 91.0% of samples correctly classified (Table 7). The FA that contributed the most were C12:0 and C14:0 (r: >0.34), associated with the presence of concentrates, and CLA *cis*-9,*trans*-11, *iso* C17:0 + C16:1 *trans*-9, C18:1 *trans*-10 + *trans*-11, and C18:2 *trans*-11,*cis*-15 (r: >0.50), associated with the absence of concentrates. Conversely, it was not possible to differentiate the relative proportion of concentrate in cow diet (only 58.3% of samples correctly classified). These latter results can be explained by the dietary composition of the C = 0 group, mainly composed of fresh herbage, given exclusively or supplemented by small amounts of conserved forages. Indeed high concentrations of all FA, which we found to be related to the absence of concentrate in cow diets, have been found in the milk of grazing cows (Ferlay et al., 2006; Chilliard et al., 2007). The discriminating performance was not improved either when the discrimination was tested on fresh herbage-based diets only or on conserved forage-based diets only (Table 7). Indeed, the response of milk FA concentrations to forage-to-concentrate ratio varies according to the type of forage and the type of concentrate (Chilliard et al., 2007; Borreani et al., 2013).

Table 6. Classification results of the discriminant analyses based on milk FA composition to authenticate conserved forage-based cow-feeding systems

Discriminant analysis	Group[1]	Total no. of samples	Correct classification in cross-validation	
			n	%
Conserved forage-feeding systems, including mixed diets	CS >50-FH = 0	26	18	69.2
	H >50-FH = 0	100	96	96.0
	GS >50-FH = 0	62	57	91.9
	MIX-FH = 0	133	101	75.9
	Total	321	272	84.7
Conserved forage-feeding systems, excluding mixed diets	CS >50-FH = 0	26	18	69.2
	H >50-FH = 0	100	96	96.0
	GS >50-FH = 0	62	57	91.9
	Total	188	171	91.0
Hay vs. grass silage	H >50-FH = 0	100	97	97.0
	GS >50-FH = 0	62	60	96.8
	Total	162	157	96.9
Hay vs. corn silage	H >50-FH = 0	100	98	98.0
	CS >50-FH = 0	26	26	100.0
	Total	126	124	98.4
Grass silage vs. corn silage	GS >50-FH = 0	62	60	96.8
	CS >50-FH = 0	26	25	96.2
	Total	88	85	96.6

[1]CS = corn silage; FH = fresh herbage; GS = grass silage; H = hay; MIX = mixed diets; numbers indicate the proportion of different feeds in the diet (on DM basis) used as threshold for the assignment of samples to the groups.

Altitude. Milk FA composition was not able to authenticate reliably the altitude origin of milk (only 73.8% of samples correctly classified). The discriminating performance remained low even when the altitude origin authentication was tested on fresh herbage- and on conserved forage-based diets only (Table 8). Similar results were obtained when using all the grouping criteria (upland zones as defined in each country, altitude sensu stricto, or removing samples of high-latitude countries where altitude threshold for upland areas is low). These results are in contrast to those reported by Engel et al. (2007), who successfully used milk FA composition to authenticate upland and lowland milk. However, their experimental design might have masked a feeding system effect beneath the altitude effect, the upland samples deriving mainly from herbage-based

Table 7. Classification results of the discriminant analyses based on milk FA composition to authenticate the level of concentrate supplementation fed to cows

Discriminant analysis	Group[1]	Total no. of samples	Correct classification in cross-validation	
			n	%
Concentrate vs. no. of concentrates	C >0	775	706	91.1
	C = 0	34	30	88.2
	Total	809	736	91.0
Level of concentrate supplementation	C0–20	305	181	59.3
	C20–30	244	124	50.8
	C >30	226	147	65.0
	Total	775	452	58.3
Level of concentrate supplementation, concerning fresh herbage >50% forage only	C0–20-FH >50	194	108	55.7
	C20–30-FH >50	62	25	40.3
	C >30-FH >50	56	39	69.6
	Total	312	172	55.1
Level of concentrate supplementation, concerning conserved forage only	C0–20-FH = 0	64	32	50.0
	C20–30-FH = 0	130	59	45.4
	C >30-FH = 0	127	76	59.8
	Total	321	167	52.0

[1]C = concentrate; FH = fresh herbage; numbers indicate the proportion of different feeds in the diet (on DM basis) used as threshold for the assignment of samples to the groups.

Table 8. Classification results of the discriminant analyses based on milk FA composition to authenticate the altitude origin of milk

Discriminant analysis	Group[1]	Total no. of samples	Correct classification in cross-validation	
			n	%
Altitude	Low	307	213	69.4
	Up	502	384	76.5
	Total	809	597	73.8
Altitude, concerning fresh herbage >50% only	Low-FH >50	79	54	68.4
	Up-FH >50	267	210	78.7
	Total	346	264	76.3
Altitude, concerning conserved forage only	Low-FH = 0	147	109	74.1
	Up-FH = 0	174	137	78.7
	Total	321	246	76.6

[1]FH = fresh herbage; Low = lowland; Up = upland; numbers indicate the proportion of different feeds in the diet (on DM basis) used as threshold for the assignment of samples to the groups.

diets and the lowland samples from diets including corn silage. Indeed, Coppa et al. (2012a) and Valenti et al. (2013) failed to authenticate the altitude origin of milk using near- or mid-IR spectroscopy. They suggested that differences in milk composition according to altitude may be due the altitude-related changes in vegetation types (De Noni and Battelli, 2008; Coppa et al., 2011), and that the differences due to altitude per se are likely small compared with those between different feeding systems (Ferlay et al., 2006, 2008; Coppa et al., 2011; Revello-Chion et al., 2010).

CONCLUSIONS

Our work used a data set of FA composition of bulk milk collected in several European countries to evaluate original and reliable models to predict cow diet composition and altitude origin. These prediction models could offer a valuable tool to authenticate cow-feeding systems. We also highlighted the effectiveness of milk FA composition to discriminate milk from different feeding systems. However, in our work, milk FA composition was not powerful enough to reliably authenticate the level of concentrate supplementation, or the upland versus lowland origin of milk. Future researchers need to include forage and concentrate characteristics (such as nutritive quality and herbage botanical composition) in the models to increase the precision of prediction and discrimination.

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