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European Food Research and Technology
Zeitschrift für Lebensmittel-Untersuchung und -Forschung A

ISSN 1438-2377

Eur Food Res Technol
DOI 10.1007/s00217-015-2473-3

Springer

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DOI 10.1007/s00217-015-2473-3

ORIGINAL PAPER

Fatty acid composition and fat content in milk from cows grazing in the Alpine region

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Received: 27 February 2015 / Revised: 1 May 2015 / Accepted: 9 May 2015

Abstract The variation in the fat profile of pooled milk from cows grazing in pastures in June and July at 400–700 m and at 1400–2250 m of altitude was evaluated by gas chromatography and compared with that from cows stalled in barns and fed with a diet without fresh grass. The ratios unsaturated/saturated fatty acid in milk samples were 1.33, 1.71 and 1.69 in June and 1.21, 1.69 and 1.84 in July for cows fed with prepared diet, grazing at 400–700 m or grazing at 1400–2250 m, respectively. Analogously, the ratios (oleic plus stearic acid)/palmitic acid were, for the same group of cows, 0.59, 0.72 and 0.78 in June and 0.56, 0.73 and 0.81 in July. In milk from pastured cows, the percentage of oleic, vaccenic, rumenic and α-linolenic acids increased as a function of the altitude; instead, that of linoleic acid and of *cis*-12-octadecenoic acid decreased. The yield of fat was always highest in milk from 1400 to 2250 m of altitude (up to 3.6 g per 100 mL). For the milk collected in July at 1400–2250 m of altitude, it was observed a decrease in the percentage of decanoic (capric) and dodecanoic acids and an increase in pentadecanoic, stearic, arachidic and docosanoic (behenic) acids. Possible reasons for the differences observed in the milk samples were discussed.

Keywords Linoleic acid · Linolenic acid · Vaccenic acid · Rumenic acid · Cow metabolism · Alps

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Introduction

Cow milk fat content and its fatty acid (FA) profile depend on numerous factors [1, 2]. They might vary because of incomplete or irregular milking, cow age (fat content increases with aging until a maximum and then it decreases), climatic conditions (dry period and temperature) [3], altitude [4] and lactation period [5] (it is lower in the first quarter after delivery and increases during lactation). Additionally, the composition of diet also influences the milk lipolytic system modifying the butyric acid and short-chain FA formation and the sensorial quality of milk during the lipolysis [6, 7]. Dietary strategy and role of forage in the diet play a crucial role for modification of milk fat content and FA composition [8–14].

Because of the potential impact on human health, FA consumption with diet has been widely studied. Milk and milk derivatives are important sources of beneficial monounsaturated FA (MUFA) and polyunsaturated FA (PUFA) [15–19]. In particular, linoleic acid (*cis*-9,*cis*-12-octadecadienoic acid), α-linolenic acid (*cis*-9,*cis*-12,*cis*-15-octadecatrienoic acid) and their positional and geometric isomers are the main constituents of PUFA. Linoleic and α-linolenic acids are the most abundant octadecadienoic and octadecatrienoic FA isomers in plants and seeds reaching concentrations as high as 50–75 % of the total lipid fraction in forage [20]. PUFA has been shown to have anticarcinogenic and antiatherogenic action [21–24], but usually the human consumption of these beneficial FA is below the dose that could be effective for reducing tumor incidence. Furthermore, the relatively high consumption of saturated fatty acids (SFA), in particular myristic (14:0) and palmitic (16:0) acids, has been associated with human cardiovascular health problems [25]. Therefore, the possibility

Published online: 20 May 2015

to increase the relative percentage of MUFA and PUFA in milk might be useful for its nutritive and healthy properties.

In the present study, the relative fraction of FA in pooled milk obtained from dairy cows grazing in pastures located in the Alps of Aosta Valley region at different altitude (400–700 and 1400–2250 m) and in two different summer months (June and July) is compared with that measured in milk samples from cows stalled in barns and fed with a prepared diet.

Materials and methods

Sampling

Milk from Alpine meadows in Aosta Valley region was collected at two different altitude ranges (400–700 and 1400–2250 m) in different farms whose cows belonged to the races of Fresian and Bruna Alpina. Milk samples were withdrawn from pooled milk of ten farms (per each range of altitude considered) in June and July with an intercurring period of time of 1 month between each sampling. In all cases, the samples of milk were frozen at −20 °C just after collection and preserved at this temperature until fat extraction.

To better distinguish the peculiarities of fat profile in milk coming from the Alpine region, analytical data were compared with those obtained from bulk milk of cows from five different farms located in Po River basin and fed with a usual prepared diet. This latter consisted in different percentages of corn silage (48–57 %), corn flour (6.3–12.5 %), barley (0–3.8 %), commercial core (9.8–14.7 %), hay of *Medicago sativa* (0–22.5 %), hay of *Gramineae* (0–5.3 %), hay of *Lolium* (0–1.9 %), cotton seeds (3.2–5.3 %), soy flour extracted (0–3.5 %), *Lolium* silage (0–1.9 %) and silage of grass from stable meadow (0–10.7 %).

Chemicals

All FA methyl esters (FAME) were obtained from Sigma-Aldrich (Milan, Italy) except the methyl ester of *cis*-9, *trans*-11-octadecadienoic acid (conjugated, rumenic acid) and of *trans*-11-octadecenoic acid (vaccenic acid) that were purchased from Nu-Check, USA. All the other reagents and solvents were of analytical grade.

Fat extraction

Lipids were extracted according to official protocol ISO14156-IDF172. Briefly, all milk samples were homogenized in a water bath at 35 °C, mixed by repeated inversion and then quickly cooled to 20 °C. Milk samples (20 mL) were mixed with 16 mL of ethanol, 4 mL of ammonia solution (30 %) and 20 mL of diethyl ether in a separating funnel, and the solutions vigorously shaken for 1 min. Then 20 mL of petroleum ether was added, and the samples were mixed carefully. After phase separation, the aqueous layers were discarded. The organic phases were treated twice with 20 mL of sodium sulfate solution (10 % in water) and then transferred into glass tubes. The samples were dried over anhydrous sodium sulfate and evaporated under reduced pressure. Recovery was evaluated by adding 40 μL of dodecane as internal standard in 20 mL of milk. The fat obtained was weighted and stored at −20 °C.

FA methyl ester preparations

FAME were prepared according to official protocol ISO15884-IDF182. Briefly, 0.2 mL of potassium hydroxide methanolic solution (2 M) was added to 5 mL of a fat extract solution (20 mg/mL) in petroleum ether. The solutions were vigorously mixed with the vortex mixer for 1 min, and after an additional reaction time of 5 min, an excess of sodium hydrogen sulfate was added. The samples were shaken again, centrifuged and after decantation were used for GC analyses.

Gas–liquid chromatography

Analyses of FAME were carried out by using an Agilent Technologies Inc. 6850 gas chromatograph (Milan, Italy), equipped with a split–splitless injector, a FID detector and a column MegaWax 25 m long, ID 0.32 mm, film thickness 0.25 μm (Mega s.n.c, Legnano, Italy). Analytical conditions are as follows: oven temperature from 40 °C (initial time 1 min) to 150 °C (10 °C/min), to 250 °C (5 °C/min) (final time 5 min); H_2 as carrier gas (flow rate 1.2 mL/min); injection temperature 300 °C; volume injected 1 μL and split ratio 1:50. Peak areas were determined by means of Software Agilent G2070 ChemStation.

Statistical analysis

Analyses were carried out in triplicate and were expressed as mean ± standard deviation (SD). Statistical differences of data were examined by using the Student's *t* test, calculated by Microsoft Excel software.

Results

Milk extracted fat is a parameter that emphasizes the differences between the milk samples. We observed that in June and in July fat content increased with the pasture altitude. Furthermore, in June and July milk produced from cows fed with a prepared diet had a slightly lower fat content compared with that obtained from grazing cows ($P < 0.05$). However, in July fat milk content was more dependent on the altitude being at 1400–2250 m about 10 and 20 % higher ($P \leq 0.01$) than that obtained at 400–700 m and from cows fed with a prepared diet, respectively (Table 1).

By GC, we obtained a satisfactory separation and quantification of the most important saturated and unsaturated FA (data not shown). In particular, octadecenoic acids as oleic acid (*cis*-9-octadecenoic acid), vaccenic acid, *cis*-12-octadecenoic acid, octadecadienoic acids, linoleic and rumenic acids, which are nutritionally desirable functional fat of milk, were detected [26, 27]. Furthermore, a baseline separated peak, assigned to methyl α-linolenate on the basis of a standard, was also monitored.

Figure 1 shows the variation in FA in milk obtained from cows fed with prepared diet, grazing at 400–700 m or milk from 1440 to 2250 m in June and in July. Concerning unsaturated FA, the percentage of oleic acid, vaccenic acid, rumenic acid and α-linolenic acid was significantly lower ($P < 0.01$) for the cows fed with a standard diet than for the cows grazing at 400–700 m or at 1440–2250 m. These latter two groups of cows also showed a concomitant decrease in the relative percentage of linoleic (18:2, *cis*-9,*cis*-12) and *cis*-12-octadecenoic acids ($P < 0.01$ in June and $P < 0.1$ in July). Interestingly, the percentage of α-linolenic acid increased as a function of the altitude and independently of the period, being 1.5 and 0.87 % in June and 1.1 and 1.5 % in July in the milk obtained from cows grazing at 1440–2250 m and 400–700 m, respectively. Analogously, also vaccenic acid had a similar trend, even though only in July, and it resulted 5.0 and 4.0 % in the milk from cows at 1440–2250 m and at 400–700 m, respectively.

With regard to saturated FA, the samples from pastured cows had a significant lower content of lauric (12:0), myristic (14:0) and palmitic acids (16:0) ($P < 0.01$ in all cases) with respect to milk from cows fed with a standard diet. Arachidic acid (20:0) and behenic acid (22:0) increased both as a function of the altitude ($P < 0.01$). No significant variation was observed for stearic acid (18:0) between the various groups. Instead, only in the case of milk obtained from cows at 1440–2250 m a change in some saturated fatty acid between June and July was observed, as shown in Table 2. In particular, a variation in shorter FA as capric (10:0) and lauric (12:0) acids and a concomitant increase in relatively longer chain acids as pentadecanoic, stearic, arachidic and behenic acids can be observed.

The ratios reported in Fig. 2 emphasize some of the differences above discussed (see legend for details).

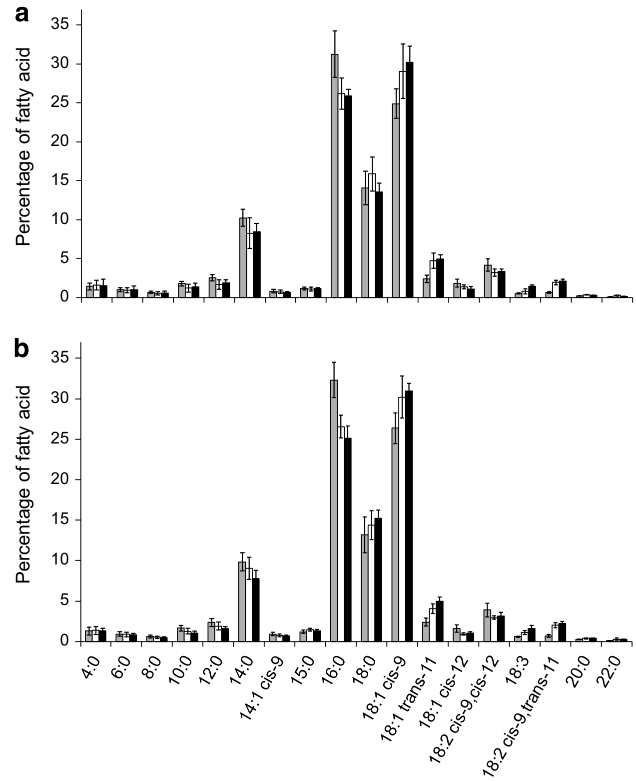

Fig. 1 Fatty acid composition of milk from cows fed with standard diet (*gray*), Alpine pasture in Aosta Valley region at altitude range of 400–700 m (*white*) and at 1400–2250 m (*black*) collected in June (**a**) and in July (**b**). (Mean of 10 determinations; *bars* correspond to the s.d.)

Table 1 Fat content in milk samples harvested at different altitudes[a]

Month	Cow fed with prepared diet[b]	400–700 m[c]	1400–2250 m[c]
June	2.9 ± 0.2	3.2 ± 0.3	3.3 ± 0.4
July	2.8 ± 0.1	3.0 ± 0.3	3.6 ± 0.3

[a] Values are reported as g of fat per 100 mL of milk ± s.d. (means obtained from 10 different samples assayed in duplicate). Percent of recovery was 94 ± 5

[b] See "Materials and methods" for the composition of prepared diet

[c] Range of altitude where milk samples were harvested

Table 2 Percentages of saturated fatty acids in milk from cows grazing at 1400–2200 m in June and July[a]

Fatty acid	June (%)	July (%)	*P* value
Capric	1.42 ± 0.43	1.05 ± 0.24	0.03
Lauric	1.91 ± 0.4	1.57 ± 0.3	0.05
Pentadecanoic	1.20 ± 0.09	1.34 ± 0.18	<0.01
Stearic	13.61 ± 1.1	15.21 ± 1.01	<0.01
Arachidic	0.31 ± 0.03	0.40 ± 0.05	<0.01
Behenic	0.18 ± 0.03	0.26 ± 0.07	<0.01

[a] Only the fatty acids whose variation had a statistical P value ≤0.05 were reported

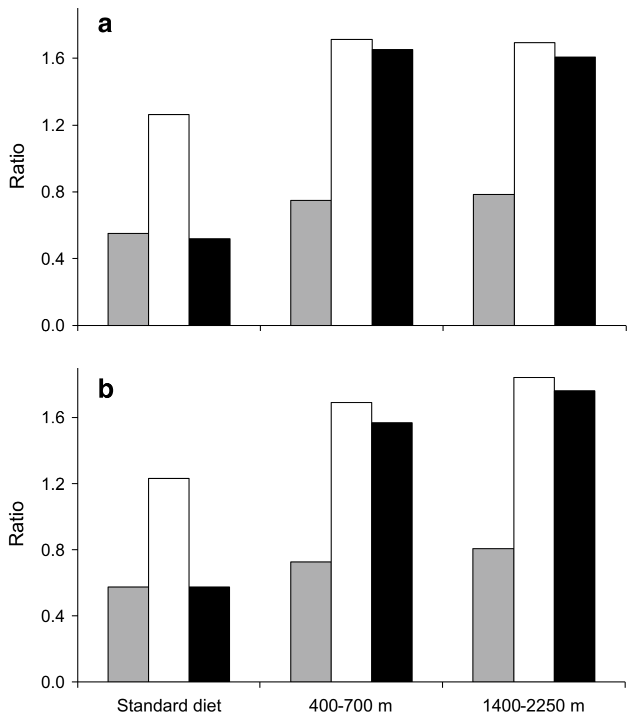

Fig. 2 Ratios (1) unsaturated/saturated FA (*gray*), (2) (stearic acid + oleic acid)/(palmitic acid) (*white*) and (3) (rumenic acid + vaccenic acid)/(linoleic acid + *cis*-12-octadecenoic acid) (*black*) for the milk samples obtained in June (**a**) or July (**b**) from cows fed with standard diet or pastured at the altitude of 400–700 or 1400–2250 m. Ratios (1) and (2) are very sensitive to variations in cow diet, and their increase is usually a desired property for milk quality improvement. Ratio (3) aims at evaluating cow metabolism with regard to biohydrogenation activity in the rumen and/or of Δ-9 desaturase activity in the epithelium of the mammary gland

Discussion

The enhancement of the levels of MUFA and PUFA is widely considered as a beneficial nutritional characteristic of food in general. The present study showed that milk obtained from cows grazing at high altitude has a fat profile with a higher content of FA as oleic acid, vaccenic acid, rumenic acid and α-linolenic acid, which are considered to improve the nutritional properties of milk and also a higher (stearic acid + oleic acid)/(palmitic acid) ratio (Fig. 2) [1, 28–30].

The routes of digestion and metabolism of dietary fatty acids in ruminant were exhaustively described and reviewed by Chilliard et al. [10], and it has been proved by numerous authors that the amount and FA profile in milk depend on the diet of the ruminant [9]. Several research groups reported a higher level of conjugated linoleic acid (CLA) in milk of cows, goats or ewes grazing fresh herb than in the milk from animals fed with dried herbage [11, 31]. In the present study, we observed that milk obtained from cows grazing in the Alpine region (pasture-fed cows) shows an increased percentage of long-chain saturated (longer than palmitic acids) and unsaturated FAs at the expense of short- and medium-chain (shorter than stearic acid) saturated FA. On the other hand, beyond a comparison of FA composition and fat content of bulk milk obtained from cows pasturing at different altitude in the Alpine region we implicitly compared the influence of supplement feeding versus pasture feeding. Therefore, in agreement with the literature [10, 32–34], it might be suggested that the increase in the unsaturated/saturated FA ratio and the differences in the fatty acid profile observed for the milk from cows grazing at different altitude (Fig. 2) mainly arises from the fresh grass or from the ripeness stage of the grass itself. In fact, the availability of sprouts of grass for a longer period of time, at 1400–2250 m compared with those at 400–700 m or with the standard diet, could also be a reason for the observed differences. Unfortunately, because of the numerous factors that contribute to determine the fat profile of milk (e.g., cow age, breed, kind of grass, etc.), it was not possible a punctual comparison with previous studies and we could not highlight if, beside pasture feeding, other facts due to the altitude (microflora, UV radiation, grass composition) could contribute to the characteristic FA profile for the milk from the Alpine region. To this end, it is worth pointing out that in a recent study carried out by Coppa et al. [35] milk FA composition was not able to authenticate reliably the altitude origin of milk (only 73.8 % of samples correctly classified). In another study, the fraction of omega-3 FA and CLA in milk from cows fed with a continuous grazing on short grass system resulted higher than that obtained from the Austrian Alpine region, and no clear differences were observed for the omega-6 FA [36].

There is still an open debate about the effects of FA on cardiovascular diseases; thus, it is difficult to establish whether these FA might improve dairy product properties [37]. However, unsaturated trans FA as vaccenic and rumenic acids also increase with the altitude. Rumenic acid is the primary octadecadienoic acid isomer (*cis*-9, *trans*-11-octadecadienoic acid), which accounts for more than 82 % of the total in dairy products [27]. Rumenic acid in milk is mainly formed from linoleic acid, which is transformed in the rumen forming vaccenic acid (*trans*-11-octadecenoic acid) by means of *Butyrivibrio fibrisolves* (biohydrogenation). Vaccenic acid can pass into milk directly or after transformation to rumenic acid by the action of Δ-9 desaturase in the epithelium of the mammary gland [10]. This metabolism could be accelerated by a higher amount of fresh grass in the diet. Buccioni et al. [38], by incubating in vitro rumen fluid acid, have found that the biohydrogenation of unsaturated FA was slower with dried herbage than with green herbage. However, because of the differences observed for milk obtained at 1400–2250 m compared with that at 400–700 m, the rumen microflora or secondary metabolites of plants which are due to the peculiar conditions found at higher altitude might play an important role [4].

Contrarily to our results, Couvreur et al. [34] showed a linear decrease in milk fat content increasing the proportion of fresh grass in the diet of cows. Therefore, on the basis of this latter indication, it can be suggested that the higher yield of fat in milk from 1400 to 2250 m (Table 1) is due to a more intense metabolism and lipid intake at this altitude.

In conclusion, the higher level of unsaturated FA and in particular of oleic acid, α-linolenic acid and trans FA (vaccenic and rumenic acids) might arise from the fresh grass in the cow diet. Nevertheless, we obtained indications that peculiar conditions present at higher altitude could also be crucial to favor the enhancement of trans FA. Through the present study, we also achieved FA profiles that could be useful to enlarge the available data that characterize the milk from Alpine region, providing parameters for the traceability systems along the whole chain of milk production.

Acknowledgments The authors are grateful to Ministero delle Politiche Agricole Alimentari e Forestali, Project IDENTILAT D.M.304/7303/05 (12/10/2005) that financially supported this publication.

Conflict of interest None.

Compliance with Ethics requirements This article does not contain any studies with human or animal subjects.

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