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ARTICLE

Winter/Spring Changes in Fatty Acid Composition of Farmhouse Idiazabal Cheese Due to Different Flock Management Systems

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Typically, two different flock managements are employed by basque sheepherders in winter and spring. Thus, seasonal changes in the fatty acid (FA) composition of Idiazabal PDO farmhouse cheeses were studied. Ewe's raw milk cheeses elaborated in winter and spring were collected after 120 days of ripening from 10 Idiazabal PDO farmhouses. In winter, concentrate and conserved forages were fed, whereas a part-time grazing system was adopted from spring onward. Spring cheeses had less ($P \leq 0.05$) saturated FA and higher ($P \leq 0.05$) content of unsaturated FA, including *trans*-FA (mainly *trans*-vaccenic acid) and conjugated linoleic acid (CLA), branched-chain FA (BCFA), and *n*-3 FA. Principal component analysis (PCA) separated winter and spring cheeses into two groups by the combination of two principal components (84.2% of variance). Fresh pasture in the diet enhanced desirable FA and lowered atherogenicity index in cheeses, supporting the benefits of using a part-time grazing system for the consumer.

KEYWORDS: Fatty acids; CLA; cheese; pasture; season; management; feed

INTRODUCTION

It is well-known that ewe's milk production is of great economic importance in Mediterranean countries where most of the milk produced is converted into cheese. A brief overall review on the cheeses manufactured in the Iberian peninsula from milk of small ruminants can be consulted in the work published by Freitas and Malcata (*1*). Idiazabal Protected Denomination of Origin (PDO) cheese is a semihard, raw milk cheese exclusively made from the milk of the *latxa* and *carranzana* breeds. It is produced in a definite geographical area that involves the Basque Country region and Navarre in northern Spain. In this area rearing animals on pastures is an ancestral tradition, which contributes to maintaining clean forests, attracting tourism, and most interestingly increasing consumer acceptance of sheep-derived high-quality products. Nowadays, the most frequently used flock management system in the Basque Country is based on concentrate and conserved forages during pasture shortage and on pastures for the rest of the year, with indoor supplementation when needed to satisfy the nutritional needs of the animals. Oregui and Falagan Prieto (*2*) reported that in the Mediterranean basin pastures tend to decrease in the farm environment and, as a consequence, in the feeding strategies, leading to the loss of the authenticity and quality of some cheeses (*3*). Thus, it is of great importance to provide scientific evidence of the advantages and benefits of pasture-based systems to encourage sheepherders not to abandon them.

Cheese composition is determined by milk composition (*4*), which in turn depends on other main production factors such as genotype, reproduction and sanitary characteristics of animals, agroclimatic conditions, and socioeconomical environment and farming methods, including feeding and milking. However, feeding is the most important one because other factors, such as season or flock sanitary status, are influenced by changes in the quantity and quality of the feeds ingested (*3*).

Milk fat is the main nutrient affected by dietary changes, and its lipid composition has gained attention in recent years because of its nutritional implication in human health. Although milk fat is highly saturated (rich in lauric, myristic, and palmitic acids), which could be related to coronary heart disease risk (*5*), other components are considered to be beneficial to human health. Among them, butyric acid, oleic acid, branched-chain fatty acids (BCFA), and polyunsaturated fatty acids (PUFA), especially *n*-3 fatty acids (*n*-3 FA) and conjugated linoleic acids (CLA), are claimed to have potential antiatherogenic, antiobesity, or anticarcinogenic roles (*6*−*8*).

In this respect, PUFA-enriched diets (including fish oils, plant oils, and seeds) have been fed to ruminants because a higher supply of these fatty acids results in lower saturated fatty acid (SFA) concentrations in milk and cheese. Fresh pasture is a good

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alternative to marine and plant oil supplements as a source of PUFA. It is especially rich in α-linolenic acid (C18:3 c9c12c15) (*7*), which is extensively biohydrogenated in the rumen to *trans*-vaccenic acid (TVA; C18:1 t11) (*6*) and further desaturated in the mammary gland to yield the naturally predominant CLA called rumenic acid (RA; C18:2 c9t11) (*9*). Hence, a healthier fatty acid profile could be achieved by integrating pasture in the diet of the flock.

Several experiments have been conducted to improve the nutritional quality of milk fat trying to enhance the level of beneficial fatty acids. Although there is a great deal of information about the impact of different supplements and diets on dairy cattle (*6*, *10*−*14*), fewer studies have been conducted on ewes (*3*, *7*, *15*), and information *in natura* under real farming conditions for this species is very scarce.

The aim of this work was to evaluate the effect of season of the year associated with changes in the management system of commercial flocks on fatty acid composition, including detailed CLA isomeric profile, in farmhouse PDO Idiazabal cheese.

MATERIALS AND METHODS

Chemicals. The following compounds were obtained as indicated: pure methyl esters of fatty acids, C4:0, C5:0, C6:0, C8:0, C9:0, C10:0, C12:0, C14:0, C16:0, C17:0, C18:0, C20:0 (HPLC or GC grade; Merck, Darmstadt, Germany); C15:0, C18:1 c9 and t9, C19:0, C18:2 t9t12, C18:2 c9t12, C18:2 c9c12, C18:3 c6c9c12, C20:1 t11, C20:1 c5, C20:1 c9, C20:1 c11, C18:3 c9c12c15, C22:0, C20:3 c8c11c14, C20:5 c5c8c11c14c17 (EPA), C22:5 c7c10c13c16c19 (DPA), C22:6 c5c7c10c13c16c19 (Sigma, Buchs, Switzerland); C18:2 c9t11, C18:2 c9c11, C18:2 t9t11, C18:2 t10c12 in acid form (Matreya Inc., Pleasant Gap, PA); C7:0, C12:1 c11, C13:0, C14:1 t9, C16:1t9, C16:1 c9, C17:1 t10, C18:1 c11, C20:2 c11c14, C20:3 c11c14c17, C20:4 c5c8c11c14 (Nu-Chek-Prep Inc., Elysian, MN); *iso*-C12:0, *anteiso*-C12:0, *iso*-C13:0, *anteiso*-C13:0, *iso*-C14:0, *anteiso*-C14:0, *iso*-C15:0, *iso*-C16:0, *iso*-C17:0, *anteiso*-C17:0 (Laordan Fine Chemicals AB, Malmö, Sweden). The methyl esters of CLA c9t11 and CLA t10c12 were obtained from Matreya Inc., and other CLA isomers were synthesized by isomerization with I_2. Solvents used for chromatography were obtained from Merck (HPLC or GC grade). All other chemicals and reagents were of analytical grade and were obtained from local suppliers.

Sampling. Ten farmhouses located in the Basque Country in northern Spain and belonging to the PDO Idiazabal cheese were selected. All farmers elaborated their cheeses with the milk from their own flocks of *latxa* breed sheep. Flock size ranged from 200 to 400 ewes as these were small factories usually run by a single family. Due to the seasonality of the milk production, cheeses are elaborated from the end of January until the middle of July. Winter cheeses and late spring cheeses, with differentiated flock management systems, were collected. A thorough standardized questionnaire about the type, quantities, and composition of the feeds supplied to each flock was completed by the farmers. Each farmhouse used different commercially available concentrate formulations and forages purchased from local suppliers. Some of the sheepherders prepared the conserved forages themselves in their farms. The nutritional label of each concentrate formulation was also collected. Information about spring feeding was partially completed by estimating the fresh pasture intake from the time spent on pasture and the rest of feeds ingested (*16*). Fodder composition data and milk and cheese yields are summarized in **Table 1**.

In winter, because good-quality fresh pastures were not available, intensive management systems based on concentrate and conserved forages were used. From spring onward, a part-time grazing system was used, which consisted of a variable time allowance on pastures and corresponding supplementation in stall during milkings. Sheep grazed both in cultivated private grasslands dominated by ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) and in other noncultivated community-owned grasslands with a higher diversity of grass species. Pastures were located at an altitude between 500 and 900 m above sea level. From a meteorological point of view, the first half of the year was very warm with high rainfall records, and weather conditions were rather similar for all locations of the flocks participating in this study. Average day temperatures of 6.9 and 14.3 °C were recorded for winter and spring, respectively. The monthly accumulated rainfall was 175.7 L/m^2 in winter and 82.4 L/m^2 in spring.

Table 1. Fodder Composition (Mean ± Standard Deviation) and Average Milk and Cheese Yields in Each Season (Winter and Spring)[a]

	winter	spring
concentrate (kg/day)	1.36 ± 0.42 a	0.86 ± 0.17 b
dry matter	1.20 ± 0.37 a	0.75 ± 0.15 b
crude protein	0.23 ± 0.06 a	0.15 ± 0.02 b
crude fiber	0.10 ± 0.06 a	0.05 ± 0.02 b
crude fat	0.04 ± 0.01 a	0.02 ± 0.01 b
ashes	0.08 ± 0.04 a	0.05 ± 0.02 b
conserved forage[b] (kg/day)	1.72 ± 1.06 a	0.20 ± 0.15 b
time on pasture[c] (h/day)	0.00 ± 0.00 a	7.06 ± 0.68 b
fresh pasture intake[c] (kg/day)	0.00 ± 0.00 a	4.56 ± 0.16 b
milk yield (L/ewe × day)	0.92 ± 0.14 a	0.50 ± 0.16 b
cheese yield (kg/L)	0.17 ± 0.01 a	0.21 ± 0.02 b

[a] Winter corresponded with intensive indoor flock management system. Spring corresponded with part-time grazing system. Means followed by different lower case letters were significantly ($P \leq 0.05$) different between spring and winter cheeses. [b] Conserved forages consisted of alfalfa and grass hay, grass silage, and maize silage. [c] Mainly cultivated grasslands with predominance of ryegrass and white clover.

Whole cheeses (∼1.5 kg/cheese) were collected directly from the ripening chambers of each farmhouse after 120 days of ripening. Winter sampling was made during February and March, and late spring sampling was made from the middle of May until the end of June. In each season (winter and spring) and farmhouse, two cheeses from the same vat were collected. A total of 20 vats were sampled, 10 from winter and 10 from late spring. Average gross compositional values reported for ripened Idiazabal cheeses throughout the manufacturing season were as follows: percentage of dry matter (DM), 68.21 ± 1.87%; total fat percentage in DM, 52.06 ± 6.21%; and total protein percentage in DM, 34.81 ± 3.98% (*17*).

Sample Preparation. Cheeses were cut in eight sections of equal weight (∼180 g). The sections were vacuum-packed and frozen at −20 °C until analysis. Two sections corresponding to different cheeses from the same vat were ground and mixed for fat extraction after removal of the rind (1.5 cm) from the portions. Therefore, a total of 20 cheese samples were prepared for fat extraction.

Fatty Acid Analysis. Fat was extracted from 10 g of ground cheese with *n*-pentane using a Soxhlet apparatus. Extracted pure fat was then dissolved in *n*-hexane, and glycerides were *trans*-esterified to the corresponding fatty acid methyl esters (FAME) by a solution of 2 M potassium hydroxide in methanol (*18*).

Fatty acid (FA) composition was analyzed in duplicate by high-resolution gas chromatography (Agilent 6890, Santa Clara, CA) with flame ionization detector as described by Collomb and Bühler (*19*). Fatty acids were separated on a CP-Sil 88 capillary column (100 m × 0.25 mm i.d. × 0.20 μm film thickness; Varian BV, Middleburg, The Netherlands) and identified on the basis of reference substances and published identifications according to the method of Collomb and Bühler (*19*). Quantification was made using *n*-nonanoic acid as internal standard. Extracted fat (0.300 g) was spiked with 5.0 mL of a 0.735 g of *n*-nonanoic acid/100 mL of *n*-hexane solution. Results were expressed as grams of FA per 100 g of fat. Unresolved compounds are reported in the text and tables as A + B (i.e., C18:1 t10 + C18:1 t11); they did not separate under the present conditions and were quantified together.

CLA isomers were analyzed in duplicate by silver ion (Ag^+)-HPLC on an Agilent LC series 1100 HPLC apparatus (Santa Clara, CA), equipped with a photodiode array detector (234 nm), using three ChromSpher Lipid columns in series (stainless steel, 25 cm × 4.6 mm i.d., 5 μm particle size, Chrompack, Middleburg, The Netherlands), according to the method of Rickert et al. (*20*), as modified by Kraft et al. (*13*). UV-grade *n*-hexane with 0.1% acetonitrile and 0.5% ethyl ether was daily prepared to use as solvent at a flow rate of 1 mL/min. The procedure described by Kraft et al. (*13*) was followed for the quantitative analysis; the amount of the unresolved GC peak corresponding to CLA t7c9, CLA t8c10, and CLA c9t11 was used as the reference amount for the sum of the HPLC peak areas of these three isomers. The amount of each CLA analyzed by HPLC

was calculated relative to this reference value. Results were expressed as milligrams of FA per gram of fat.

The above-described GC and (Ag^+)-HPLC methods have been previously used to determine the fatty acid composition of ewe's milk fat by Collomb et al. (*21*).

Statistical Analysis. The SPSS statistical package, version 16.0 (SPSS Inc., Chicago, IL), was used for the statistical analyses. Analysis of variance (ANOVA) was used to determine the presence of significant differences ($P \leq 0.05$) in the analytical variables between cheeses from winter-feeding flocks and spring-feeding flocks. A general linear model was used including "season" as fixed effect and "factory" as random effect. *F* test of the "season" against the interaction term "season × flock" was used to determine significant differences. Principal component analysis (PCA) was performed on a matrix of the flock feeding variables and selected FA groups having communality values higher than 0.4. The Kaiser criterion (eigenvalue > 1) was used to select the principal components. Factors were rotated (Varimax method) for ease of interpretation.

RESULTS AND DISCUSSION

Milk and Cheese Yields. Milk yield and cheese yield records corresponding to each season are reported in **Table 1**. In late spring, when the cheeses from part-time grazing systems were elaborated, the animals were in their late-lactation phase and, accordingly, milk yield was lower ($P \leq 0.05$) (*22*). Late-lactation milk has higher dry matter content than the milk from early lactation and, as a consequence, cheese yield was higher ($P \leq 0.05$) in spring than in winter. In seasonal calving systems, the effects of stage of lactation are confounded with those of season, that is, the effects of variation in photoperiod, weather, and diet. Because this study was conducted on commercial flocks under real farming conditions, it was not possible to compare the indoor intensive management and the part-time grazing management in the same season because they never coexist at the same time. However, Perojo et al. (*16*), observed very poor differences in milk yield when comparing animals with normal access to pasture under the dairy *latxa* production system and animals with restricted access to pasture and higher indoor supplement intake. These differences were nonexistent in terms of standard milk production, suggesting that replacement of pasture with indoor supplementation might not result in higher cheese yields. Future studies on experimental flocks with well-controlled model systems would help in understanding the effect of each single factor.

Groups of Fatty Acids. Mean concentrations of FA groups in winter and spring cheeses are summarized in **Table 2**. Compared to winter cheeses, spring cheeses had significantly ($P \leq 0.05$) lower concentrations of SFA (8.6% lower). The decrease in SFA content in spring cheeses was basically due to the decrease in short-chain fatty acids (SCFA, 21.8% lower) and, to a lesser extent, in medium-chain fatty acids (MCFA, 8.6% lower). On the contrary, higher concentrations ($P \leq 0.05$) of long-chain fatty acids (LCFA, 24.3% higher), monounsaturated fatty acids (MUFA, 27.1% higher), and PUFA (18.8% higher), CLA (59.4% higher), and *trans*-FA without CLA (49.6% higher) were found in cheeses from part-time grazing systems (spring cheeses) than in those produced during the winter months. The content of BCFA and *n*-3 FA also increased ($P \leq 0.05$) in spring cheeses (12.2 and 21.4% higher, respectively), whereas the *n*-6 FA content and *n*-3/*n*-6 ratio did not differ significantly ($P > 0.05$) between winter and spring cheeses (**Table 2**).

As a result of pasture-based feeding in spring, unsaturated fatty acid (UFA) content increased ($P \leq 0.05$) to the detriment of SFA content in the cheeses. This led to a 1.5-fold decrease in the atherogenicity index of the cheese fat, defined as (C12:0 + (4 × C14:0) + C16:0)/UFA (*23*), resulting in a healthier fatty acid composition of cheeses from grazing flocks (**Table 2**). A similar result was previously observed in milk fat of ewes (*15*) and lactating cows (*12*, *14*) reared on grazing systems compared to indoor feeding based on concentrate and conserved forages.

SCFA and MCFA accounted for 88.1 and 83.6% of the total SFA in winter and spring cheeses, respectively (**Table 2**). The SCFA and MCFA groups generate from de novo synthesis in the mammary gland by acetyl CoA carboxylase and fatty acid synthase, and most of them are saturated because the Δ9-desaturase activity is very low when fatty acid chain length is shorter than 18 carbons (*6*). Palmquist et al. (*10*) suggested that PUFA-rich diets inhibit de novo synthesis of fatty acids due to a

Table 2. Concentrations (Grams per 100 g of Fat, Mean ± Standard Deviation) of Fatty Acid (FA) Groups in Winter and Spring Cheeses

	winter	spring
significantly ($P \leq 0.05$) higher in winter		
short-chain FA[a]	17.252 ± 0.985	13.487 ± 2.060
medium-chain FA[b]	41.735 ± 2.046	38.132 ± 3.141
saturated FA[c]	65.484 ± 2.253	59.838 ± 2.966
atherogenicity index[d]	3.446 ± 0.207	2.349 ± 0.386
significantly ($P \leq 0.05$) higher in spring		
long-chain FA[e]	26.465 ± 1.712	34.975 ± 4.261
C18:1 FA[f]	14.482 ± 0.984	19.976 ± 2.499
C18:2 FA[g]	3.078 ± 0.461	4.000 ± 0.536
unsaturated FA[h]	19.822 ± 0.998	26.622 ± 2.640
monounsaturated FA[i]	15.739 ± 1.020	21.593 ± 2.443
polyunsaturated FA[j]	4.083 ± 0.491	5.028 ± 0.575
trans-C18:1 FA[k]	2.185 ± 0.486	4.280 ± 1.267
conjugated linoleic acids[l]	0.402 ± 0.124	0.989 ± 0.270
trans-FA without conjugated linoleic acids[m]	2.829 ± 0.555	5.615 ± 1.634
branched-chain FA[n]	1.827 ± 0.131	2.079 ± 0.189
n-3 FA[o]	0.934 ± 0.130	1.188 ± 0.269
without significant ($P > 0.05$) differences		
n-6 FA[p]	2.992 ± 0.424	2.988 ± 0.250
n-3 FA/*n*-6 FA	0.372 ± 0.084	0.297 ± 0.072

[a] C4:0, C5:0, C6:0, C7:0, C8:0, C10:0, C10:1. [b] C12:0, *iso*-C13:0, *anteiso*-C13:0, C12:1 c9 + C13:0, *iso*-C14:0, C14:0, *iso*-C15:0, C14:1 t9, *anteiso*-C15:0, C14:1 c9, C15:0, *iso*-C16:0, C16:0, *iso*-C17:0, C16:1 t9, *anteiso*-C17:0, C16:1 c9. [c] C4:0, C5:0, C6:0, C7:0, C8:0, C10:0, C12:0, branched-chain FA, C14:0, C15:0, C16:0, C17:0, C18:0, C19:0, C20:0, C22:0. [d] (C12:0 + (4 × C14:0) + C16:0)/unsaturated FA. [e] C17:0, *iso*-C18:0, C17:1 t10, *anteiso*-C18:0, C18:0, C18:1 FA, C19:0, C18:2 FA, C20:0, C20:1 t11, C18:3 c6c9c12, C20:1 c5, C20:1 c9, C20:1 c11, C18:3 c9c12c15, C20:2 c11c14, C22:0, C20:3 c8c11c14, C20:3 c11c14c17, C20:4 c5c8c11c14, C20:5 c5c8c11c14c17 (EPA), C22:5 c7c10c13c16c19 (DPA), C22:6 c5c7c10c13c16c19 (DHA). [f] C18:1 t4, C18:1 t5, C18:1 t6 + C18:1 t7 + C18:1 t8, C18:1 t9, C18:1 t10 + C18:1 t11, C18:1 t12, C18:1 t13 + C18:1 t14 + C18:1 c6 + C18:1 c7 + C18:1 c8, C18:1 c9, C18:1 c11, C18:1 c12, C18:1 c13, C18:1 t16 + C18:1 c14. [g] C18:2 t,tNMID, C18:2 t9t12, C18:2 c9t13 + C18:2 t8c12, C18:2 c9t12 + C18:2 c,cMID + C18:2 t8c13, C18:2 t11c15 + C18:2 t9c12, C18:2 c9c12, C18:2 c9c15, C18:2 c9t11 + C18:2 t8c10 + C18:2 t7c9, C18:2 t11c13 + C18:2 c9c11, C18:2 t9t11. [h] C10:1, C14:1 t9, C14:1 c9, C16:1 t9, C16:1 c9, C17:1 t10, C18:1 FA, C20:1 t11, C20:1 c5, C20:1 c9, C20:1 c11, C18:2 FA, C18:3 c6c9c12, C18:3 c9c12c15, C20:2 c11c14, C20:3 c8c11c14, C20:3 c11c14c17, C20:4 c5c8c11c14, C20:5 c5c8c11c14c17 (EPA), C22:5 c7c10c13c16c19 (DPA), C22:6 c5c7c10c13c16c19 (DHA). [i] C10:1, C14:1 t9, C14:1 c9, C16:1 t9, C16:1 c9, C17:1 t10, C18:1 FA, C20:1 t11, C20:1 c5, C20:1 c9, C20:1 c11. [j] C18:2 FA, C18:3 c6c9c12, C18:3 c9c12c15, C20:2 c11c14, C20:3 c8c11c14, C20:3 c11c14c17, C20:4 c5c8c11c14, C20:5 c5c8c11c14c17 (EPA), C22:5 c7c10c13c16c19 (DPA), C22:6 c5c7c10c13c16c19 (DHA). [k] C18:1 t4, C18:1 t5, C18:1 t6 + C18:1 t7 + C18:1 t8, C18:1 t9, C18:1 t10 + C18:1 t11, C18:1 t12, C18:1 t13 + C18:1 t14 + C18:1 c6 + C18:1 c7 + C18:1 c8. [l] C18:2 c9t11 + C18:2 t8c10 + C18:2 t7c9, C18:2 t11c13 + C18:2 c9c11, C18:2 t9t11. [m] C14:1 t9, C16:1 t9, C17:1 t10, C20:1 t11, *trans*-C18:1 FA, C18:2 t,tNMID, C18:2 t9t12, C18:2 c9t13 + C18:2 t8c12, C18:2 c9t12 + C18:2 c,cMID + C18:2 t8c13, C18:2 t11c15 + C18:2 t9c12. [n] *iso*-C13:0, *anteiso*-C13:0, *iso*-C14:0, *iso*-C15:0, *anteiso*-C15:0, *iso*-C16:0, *iso*-C17:0, *anteiso*-C17:0, *iso*-C18:0, *anteiso*-C18:0. [o] C18:2 t11c15 + C18:2 t9c12, C18:2 c9c15, C18:3 c9c12c15, C20:3 c11c14c17, C20:5 c5c8c11c14c17 (EPA), C22:5 c7c10c13c16c19 (DPA), C22:6 c5c7c10c13c16c19 (DHA). [p] C18:1 t12, C18:1 c12, C18:2 t9t12, C18:2 c9t12 + C18:2 c,cMID + C18:2 t8c13, C18:2 c9c12, C18:3 c6c9c12, C20:2 c11c14, C20:3 c8c11c14, C20:4 c5c8c11c14. c, *cis*; t, *trans*; NMID, non-methylene-interrupted diene; MID, methylene interrupted diene.

greater uptake and secretion of dietary and ruminally derived fatty acids. These fatty acids would compete for esterification with SCFA synthesized in the mammary gland, leading to a feedback inhibition of the two lipogenic enzymes. High levels of LCFA have also a direct inhibitory effect on acetyl CoA carboxylase as reported by Chilliard et al. (*6*).

Individual Fatty Acids. Mean contents of individual fatty acids in winter and spring cheeses are shown in **Table 3**. Compared to cheeses made in winter, cheeses made in spring had significantly ($P \leq 0.05$) lower contents of butyric (C4:0), caproic (C6:0), caprylic (C8:0), capric (C10:0), lauric (C12:0), myristic (C14:0), and palmitic (C16:0) acids, which accounted for 84 and 78.6% of the total SFA content in winter and spring cheeses, respectively. The content of linoleic acid (C18:2 c9c12) also decreased ($P \leq 0.05$) in spring cheeses (11.7% lower than in winter) (**Table 3**). Conversely, *iso*-C17:0 and most *anteiso*-BCFA, stearic acid (C18:0), oleic acid (C18:1 c9), most C18:1 FA including *trans*-vaccenic acid (TVA, C18:1 t11), and most C18:2 FA including predominant CLA isomers and docosahexaenoic acid (DHA, C22:6 c5c7c10c13c16c19) presented higher concentrations ($P \leq 0.05$) in spring cheeses than those found in winter cheeses. No significant differences ($P > 0.05$) were observed for the concentrations of most *iso*-BCFA, most long-chain *n*-6 FA, α-linolenic acid (C18:3 c9c12c15), and other long-chain *n*-3 FA such as C20:3 c11c14c17, eicosapentaenoic acid (EPA, C20:5 c5c8c11c14c17), and docosapentaenoic acid (DPA, C22:5 c7c10c13c16c19) (**Table 3**).

Fresh pasture is the main source of α-linolenic acid (*7*, *14*). Many authors have reported higher concentrations of this fatty acid in milk from grazing animals than in milk from animals fed concentrate and conserved forages in stall (*11*, *15*). Although higher values of this fatty acid would have been valuable from a nutritional point of view, no significant increase was observed in the present study for this fatty acid in spring cheeses despite the presumably higher intake of α-linolenic acid by grazing flocks. Doreau et al. (*24*) reported extensive ruminal biohydrogenation rates for unsaturated fatty acids, almost complete for α-linolenic acid and between 60 and 95% for linoleic acid. Kucuk et al. (*25*) observed a linear increase in the biohydrogenation rate of oleic, linoleic, and α-linolenic acids as forage level in the diet increased, being much more extensive for linoleic and α-linolenic (around 90−96%) than for oleic acid. In 1966 Wilde and Dowson (*26*) described the biohydrogenation pathway of α-linolenic acid (C18:3 c9c12c15) that comprised a first isomerization step to C18:3 c9t11c15, followed by the reduction of the double bonds at positions 9, 15, and 11, yielding C18:2 t11c15, C18:1 t11, and C18:0, in this order. This could explain the fact that the α-linolenic acid content remained stable in both winter and spring cheeses and the accumulation in spring cheeses of stearic acid (C18:0) and other biohydrogenation intermediates, such as C18:2 t11c15 + C18:2 t9c12 and *trans*-C18:1 FA, especially C18:1 t10 + C18:1 t11. This last peak was 2.4 times higher in cheese made from milk of pasture-fed ewes than in cheese made in winter (**Table 3**). Indeed, in a study conducted on dairy farms with differentiated winter and spring feeding managements similar to those presented in this paper (*27*), the strong increase of TVA (C18:1 t11) and C18:2 t11c15 and the modest increase of α-linolenic acid (C18:3 c9c12c15) in spring cheeses was attributed to the differences in ruminal biohydrogenation activity. Collomb et al. (*28*) also found higher levels of C18:1 t10 + t11 and C18:2 t11c15 + t9c12 in milk from organic farming, in which animals were fed lower amounts of concentrate and higher levels of feed grasses, than in conventional integrated farming. In addition to this, because the α-linolenic acid content of fresh grass depends on environmental factors such as rainfall and light exposure and the maturity stage of green plants, as well as grass variety, it is understandable that pasture does not always increase the percentage of α-linolenic acid in milk fat (*4*, *6*, *7*).

Another major microbial transformation in the rumen is the synthesis of odd- and branched-chain fatty acids, which are important components of microbial lipids with potential anticancer activity and are not present in feeds (*29*). Amylolytic bacteria show low levels of BCFA compared to cellulolytic bacteria, which have higher content of *iso*- and *anteiso*-FA (*8*). Part-time grazing systems adopted by sheepherders in the spring entailed an increase in forage/concentrate ratio and a presumably higher dietary crude fiber intake that generally promotes the cellulolytic bacteria in the rumen (*8*). This is in good agreement with the results reported in this work because predominant BCFA, which were *anteiso*-C15:0 and *iso*- and *anteiso*-C17:0, had significantly higher concentrations in spring when fresh grass was present in the diet (**Table 3**).

Only a few studies have reported the concentrations of fatty acids of 20 carbon atoms or longer in milk fat (*28*, *30*) and sheep's milk cheese (*4*). Among them, *n*-3 FA, specifically DHA and EPA, are the most interesting ones because they can exert antithrombotic and antiarrhythmic properties (*31*), and DHA is considered to be essential for the development and maintenance of the brain, retina, and nerves (*32*). Humans convert very little linoleic acid to EPA and DHA. Therefore these fatty acids have to be supplied through the diet. Although cheeses from pasture-fed ewes had a significantly ($P \leq 0.05$) higher content of DHA than those from winter feeding, both EPA and DHA levels in this work were slightly lower than those reported by Nudda et al. (*4*). Overall, EPA and DHA constituted 0.089% and 0.095% of the total fatty acids in winter and spring cheeses, respectively (**Table 3**). However, these values are very far from those reported for ruminant milk fat when fish oil supplements were used in the diet (*6*).

***trans*- Fatty Acids without CLA.** *trans*-FA content of spring cheeses doubled as a result of pasture-based spring feeding. Quantitatively, *trans*-C18:1 FA was the most important group, comprising 2.56% of total fatty acids in winter cheeses and 4.94% in spring cheeses (**Table 2**). The concentration of each *trans*-FA peak was always significantly ($P \leq 0.05$) higher in spring cheeses than in winter cheeses (**Table 3**). The content of *trans*-FA in the cheeses and the proportions of different *trans*-C18:1 FA isomers were close to the ranges published by Goudjil et al. (*33*) for ewe′s milk fat, except for elaidic acid (C18:1 t9) and C18:1 t12, which were higher in this work. As mentioned earlier, the greatest increase was observed for the C18:1 t10 + C18:1 t11 peak, accounting for around half of the total *trans*-C18:1 FA (44.7 and 54.4% in winter and spring cheeses, respectively). Although it was not possible to resolve the compounds of this peak, based on the published work about the accumulation of TVA (C18:1 t11) produced during the fermentation of PUFA in the rumen, as discussed above, it could be suggested that it is likely to be responsible for the great increase of this peak in spring cheeses (**Table 3**).

The distribution of *trans*-C18:1 FA isomers could be of great nutritional concern, because avoidance of dairy products is frequently recommended for people who wish to limit their *trans*-fat intake. Although a high intake of *trans*-MUFA has been associated with coronary heart disease risk and myocardial infarction, *trans*-FA from dairy products and those from partially hydrogenated vegetable oils seem to have different effects on that risk (*34*). Most hydrogenated vegetable oils are enriched in C18:1 t9 and C18:1 t10, elaidic acid (*trans*-9 isomer) being the most widely studied in regard to coronary heart disease risk. On the contrary, TVA (C18:1 t11), the main natural isomer in dairy

Table 3. Concentration (Grams per 100 g of Fat, Mean ± Standard Deviation) of Individual Fatty Acids in Winter and Spring Cheeses[a]

		winter	spring
significantly ($P \leq 0.05$) higher in winter			
butyric acid	C4:0	3.100 ± 0.202	2.750 ± 0.165
caproic acid	C6:0	2.694 ± 0.115	2.169 ± 0.252
caprylic acid	C8:0	2.722 ± 0.186	2.071 ± 0.359
capric acid	C10:0	8.340 ± 0.739	6.149 ± 1.391
lauric acid	C12:0	4.703 ± 0.413	3.642 ± 0.998
myristic acid	C14:0	10.009 ± 0.471	9.263 ± 0.965
palmitic acid	C16:0	23.426 ± 1.486	20.990 ± 1.501
α-linoleic acid	C18:2 c9c12	2.130 ± 0.328	1.876 ± 0.189
t11-eicosenoic acid	C20:1 t11	0.066 ± 0.012	0.057 ± 0.011
c11-eicosenoic acid	C20:1 c11	0.046 ± 0.009	0.052 ± 0.009
significantly ($P \leq 0.05$) higher in spring			
anteiso-pentadecanoic acid	*anteiso*-C15:0	0.402 ± 0.036	0.480 ± 0.051
myristoleic acid	C14:1 c9	0.133 ± 0.015	0.193 ± 0.051
iso-heptadecanoic acid	*iso*-C17:0	0.324 ± 0.031	0.393 ± 0.033
palmitelaidic acid	C16:1 t9	0.061 ± 0.017	0.163 ± 0.061
anteiso-heptadecanoic acid	*anteiso*-C17:0	0.379 ± 0.034	0.427 ± 0.056
palmitoleic acid	C16:1 c9	0.595 ± 0.073	0.804 ± 0.143
t10-heptadecenoic acid	C17:1 t10	0.013 ± 0.001	0.016 ± 0.006
anteiso-octadecanoic acid	*anteiso*-C18:0	0.016 ± 0.006	0.028 ± 0.010
stearic acid	C18:0	6.885 ± 0.967	8.869 ± 2.008
t4-octadecenoic acid	C18:1 t4	0.020 ± 0.005	0.027 ± 0.009
t5-octadecenoic acid	C18:1 t5	0.013 ± 0.002	0.018 ± 0.007
unresolved 1	C18:1 t6−8	0.108 ± 0.028	0.169 ± 0.055
elaidic acid	C18:1 t9	0.174 ± 0.018	0.238 ± 0.042
unresolved 2	C18:1 t10 + t11 (TVA)	0.976 ± 0.304	2.329 ± 0.969
t12-octadecenoic acid	C18:1 t12	0.203 ± 0.048	0.314 ± 0.055
unresolved 3	C18:1 t13−14 + c6−8	0.446 ± 0.083	0.781 ± 0.238
oleic acid	C18:1 c9	11.550 ± 1.245	14.814 ± 2.618
vaccenic acid	C18:1 c11	0.434 ± 0.056	0.541 ± 0.078
c13-octadecenoic acid	C18:1 c13	0.061 ± 0.009	0.086 ± 0.014
unresolved 4	C18:1 t16 + c14	0.248 ± 0.039	0.404 ± 0.052
t,t-NMID-octadecadienoic acid	ΣC18:2 t,t-NMID	0.043 ± 0.009	0.101 ± 0.029
unresolved 5	C18:2 c9t13 + t8c12	0.167 ± 0.026	0.330 ± 0.100
unresolved 6	C18:2 c9t12 + c,c-MID + t8c13	0.216 ± 0.024	0.332 ± 0.049
unresolved 7	C18:2 t11c15 + t9c12	0.078 ± 0.017	0.300 ± 0.176
c9,c15-octadecadienoic acid	C18:2 c9c15	0.032 ± 0.005	0.043 ± 0.008
gadoleic acid	C20:1 c9	0.024 ± 0.003	0.033 ± 0.007
unresolved 8	C18:2 c9t11 + t8c10 + t7c9	0.357 ± 0.123	0.913 ± 0.257
unresolved 9	C18:2 t11c13 + c9c11	0.013 ± 0.003	0.034 ± 0.011
behenic acid	C22:0	0.041 ± 0.007	0.083 ± 0.018
docosahexaenoic acid (DHA)	C22:6 c5c7c10c13c16c19	0.030 ± 0.006	0.036 ± 0.007
without significant ($P > 0.05$) differences			
valeric acid	C5:0	0.034 ± 0.009	0.034 ± 0.004
enanthic acid	C7:0	0.035 ± 0.006	0.028 ± 0.012
caproleic acid	C10:1	0.327 ± 0.043	0.286 ± 0.069
iso-tridecanoic acid	*iso*-C13:0	0.030 ± 0.031	0.026 ± 0.006
anteiso-tridecanoic acid	*anteiso*-C13:0	0.041 ± 0.006	0.045 ± 0.014
unresolved 10	C12:1 c11 + C13:0	0.146 ± 0.023	0.134 ± 0.046
myristelaidic acid	*iso*-C14:0	0.111 ± 0.014	0.111 ± 0.014
iso-pentadecanoic acid	*iso*-C15:0	0.220 ± 0.033	0.257 ± 0.053
t9-tetradecenoic acid	C14:1 t9	0.010 ± 0.001	0.011 ± 0.002
pentadecanoic acid	C15:0	0.896 ± 0.085	0.937 ± 0.992
iso-hexadecanoic acid	*iso*-C16:0	0.260 ± 0.019	0.261 ± 0.024
margaric acid	C17:0	0.488 ± 0.065	0.471 ± 0.045
iso-octadecanoic acid	*iso*-C18:0	0.047 ± 0.009	0.052 ± 0.010
c12-octadecenoic acid	C18:1 c12	0.252 ± 0.048	0.255 ± 0.035
nonadecanoic acid	C19:0	0.102 ± 0.013	0.087 ± 0.016
linoelaidic acid	C18:2 t9t12	0.014 ± 0.004	0.029 ± 0.027
arachidic acid	C20:0	0.183 ± 0.028	0.216 ± 0.041
γ-linolenic acid	C18:3 c6c9c12	0.015 ± 0.002	0.015 ± 0.002
c5-eicosenoic acid	C20:1 c5	0.010 ± 0.001	0.012 ± 0.003
α-linolenic acid	C18:3 c9c12c15	0.647 ± 0.112	0.656 ± 0.141

Table 3. Continued

		winter	spring
t9,t11-octadecadienoic acid	C18:2 t9t11	0.034 ± 0.017	0.042 ± 0.020
eicosadienoic acid	C20:2 c11c14	0.019 ± 0.003	0.019 ± 0.003
homo-γ-linolenic acid	C20:3 c8c11c14	0.022 ± 0.004	0.022 ± 0.002
c8,c11,c14-eicosatrienoic acid	C20:3 c11c14c17	0.011 ± 0.001	0.011 ± 0.001
arachidonic acid	C20:4 c5c8c11c14	0.127 ± 0.017	0.1129 ± 0.013
eicosapentaenoic acid (EPA)	C20:5 c5c8c11c14c17	0.046 ± 0.006	0.047 ± 0.007
docosapentaenoic acid (DPA)	C22:5 c7c10c13c16c19	0.097 ± 0.026	0.103 ± 0.015

[a] c, *cis*; t, *trans*; TVA, *trans*-vaccenic acid; NMID, non-methylene-interrupted diene; MID, methylene-interrupted diene. Unresolved peaks: 1, petroselaidic acid + t7-octadecenoic acid + t8-octadecenoic acid; 2, t10-octadecenoic acid + *trans*-vaccenic acid; 3, t13-octadecenoic acid + t14-octadecenoic acid + c6-octadecenoic acid + c7-octadecenoic acid + c8-octadecenoic acid; 4, t16-octadecenoic acid + c14-octadecenoic acid; 5, c9,t13-octadecadienoic acid + t8,c12-octadecadienoic acid; 6, c9,t12-octadienoic acid + c,c-MID-octadecadienoic acid + t8,c13-octadecadienoic acid; 7, t11,c15-octadecadienoic acid + t9,c12-octadecadienoic acid; 8, rumenic acid + t8,c10-octadecadienoic acid + t7,c9-octadecadienoic acid; 9, t11,c13-octadecadienoic acid + c9,c11-octadecadienoic acid; 10, c11-dodecenoic acid + tridecanoic acid.

Table 4. Concentrations (Milligrams per Gram of Fat, Mean ± Standard Deviation) of Conjugated Linoleic Acid (CLA) Isomers in Winter and Spring Cheeses

	winter	spring
significantly ($P \leq 0.05$) higher in spring		
C18:2 t7,c9	0.26 ± 0.08	0.43 ± 0.13
C18:2 t12,t14	0.09 ± 0.02	0.20 ± 0.04
C18:2 t11,t13	0.10 ± 0.03	0.35 ± 0.09
C18:2 t9,t11	0.13 ± 0.02	0.21 ± 0.05
C18:2 c,t/t,c12, 14	0.05 ± 0.02	0.10 ± 0.03
C18:2 t11,c13	0.08 ± 0.02	0.29 ± 0.10
C18:2 c9,t11	3.15 ± 1.13	8.44 ± 2.41
C18:2 t8,c10	0.15 ± 0.04	0.26 ± 0.06
CLA t,t[a]	0.48 ± 0.08	0.96 ± 0.18
CLA c,t/t,c[b]	3.48 ± 1.20	9.16 ± 2.54
CLA (total)	3.96 ± 1.25	10.11 ± 2.61
without significant ($P > 0.05$) differences		
C18:2 t10,t12	0.03 ± 0.01	0.04 ± 0.01
C18:2 t8,t10	0.02 ± 0.01	0.04 ± 0.05
C18:2 t7,t9	0.06 ± 0.02	0.06 ± 0.02
C18:2 t6,t8	0.04 ± 0.02	0.04 ± 0.01
C18:2 c11,t13	0.01 ± 0.01	0.03 ± 0.04
C18:2 t10,c12	0.04 ± 0.01	0.04 ± 0.02

[a] t,t, *all-trans*-CLA. [b] c,t/t,c, CLA containing cis- and trans-double bonds.

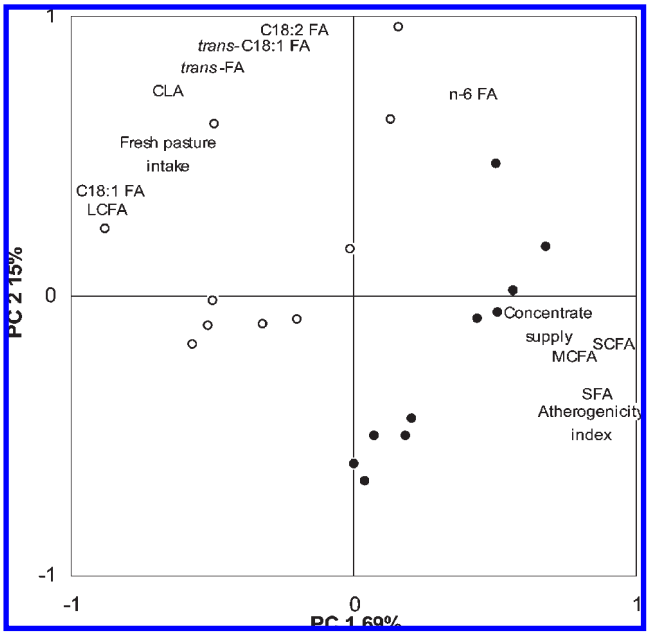

Figure 1. Plot depicting variable loadings and cheese sample distribution in the two-dimensional coordinate system defined by PC1 and PC2: winter cheeses (●); spring cheeses (○). CLA, conjugated linoleic acid; FA, fatty acids; LCFA, long-chain fatty acids; MCFA, medium-chain fatty acids; SCFA, short-chain fatty acids; SFA, saturated fatty acids.

products and presumably in the studied cheeses as well, plays a crucial role as precursor of rumenic acid (RA, CLA c9t11), which is the isomer generally credited with anticarcinogenic and antiatherogenic activities (*35*). Furthermore, Kuhnt et al. (*36*) stated that approximately one-fourth of dietary TVA was endogenously converted to RA by humans, suggesting that TVA should be taken into account when the total CLA supply in human diet is determined.

CLA Isomers. Average values of individual CLA isomer concentrations for winter and spring cheeses are displayed in **Table 4**. As a result of taking sheep out to pastures, CLA content in spring cheeses underwent a 2.5-fold increase. The concentration of the quantitatively main CLA isomers was significantly higher in spring cheeses than in winter cheeses. RA (CLA c9t11) was the predominant CLA isomer, followed by CLA t7c9. This is consistent with the relative importance of the endogenous synthesis by the Δ9-desaturase of RA from TVA, and, to a lesser extent, of CLA t7c9 from C18:1 t7 as suggested by Chilliard et al. (*6*). Several authors have reported a positive association between fresh pasture feeding and CLA t11t13 and CLA t11c13, which could be released by biohydrogenation of α-linolenic acid (*6*, *27*, *28*).

Principal Component Analysis. PCA was applied to feeding management variables (concentrate supply and fresh pasture intake), atherogenicity index, and selected FA groups (SCFA, MCFA, LCFA, SFA, C18:1 FA, C18:2 FA, CLA, *trans*-FA, *trans*-C18:1 FA, and *n*-6 FA). **Figure 1** depicts variable loadings and cheese sample scores in the two-dimensional coordinate system defined by PC1 and PC2. These two PCs accounting for 84.2% of the total variance described the variation of FA composition of cheeses elaborated in winter and spring under two clearly differentiated feeding management practices (**Table 1**).

Feed variables showed high loadings (≥|0.72|) with PC1, although the correlation value was positive for concentrate supply and negative for fresh pasture intake. SCFA, MCFA, SFA, and atherogenicity index had high positive loadings (≥0.80) with PC1, whereas LCFA, C18:1 FA, and CLA showed negative loadings (≥|0.63|) with this factor (**Figure 1**). Therefore, concentrate supply was positively associated with the increment of saturated FA content, whereas fresh pasture intake was responsible for the increment of unsaturated FA content in cheese. This factor was defined as "feeding management factor". C18:2 FA, CLA, *trans*-C18:1 FA, *trans*-FA, and *n*-6 FA showed high positive loadings (≥0.71) with PC2. Pasture intake also contributed to this component with a positive loading of 0.52. As has

been discussed before, these groups of FA are involved in biohydrogenation processes, which may be altered by the presence of fresh grass in the diet (*6*, *25*). Accordingly, this component was defined as "biohydrogenation factor".

Cheese samples from winter or spring season were clearly distinguished by the combination of the feeding management factor (PC1) and the biohydrogenation factor (PC2) (**Figure 1**). Samples located in the lower right-hand area of the graph (winter cheeses) presented a higher content of saturated fat, whereas samples located in the upper left-hand area (spring cheeses) had a higher content of unsaturated fat. The variability observed in the scores within each season was most likely due to small differences in flock management among the farmhouses collaborating in this work.

In summary, cheeses elaborated with milk from ewes fed fresh pasture contained less saturated and atherogenic fat and had higher levels of nutritionally desirable fatty acids, such as RA, TVA, DHA, and BCFA, in comparison with cheeses elaborated with milk from the same ewes in intensive farming systems based on concentrate and conserved forages. Because per capita cheese consumption in Europe is ~18.5 kg per year (*37*), the contribution of cheese fat to human diet is significant. The region in which this study took place is located within the Mediterranean countries, where ewe′s cheese consumption is of great importance (*1*). Thus, data on cheese fat composition reported in this work will contribute to provide scientific evidence on the advantages of pasture-based systems to obtain high-quality products and to encourage sheepherders to improve and continue part-time grazing management.

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LITERATURE CITED

(1) Freitas, C.; Malcata, F. X. Microbiology and biochemistry of cheeses with appélation d′Origine Protegée and manufactured in the Iberian peninsula. *J. Dairy Sci*. **1999**, *83*, 584–602.

(2) Oregui, L. M.; Falagan Prieto, A. Spécificités et diversité des systèmes de production ovine et caprine dans le Bassin Méditerranéen (Specificity and diversity of sheep and goat production systems in Mediterranean Basin). *Options Méditerranéennes, A* **2006**, *70*, 77–86.

(3) Morand-Fehr, P.; Fedele, V.; Decandia, M.; Le Frileux, Y. Influence of farming and feeding systems on composition and quality of goat and sheep milk. *Small Ruminant Res*. **2007**, *68*, 20–34.

(4) Nudda, A.; McGuire, M. A.; Battacone, G.; Pulina, G. Seasonal variation in conjugated linoleic acid and vaccenic acid in milk fat of sheep and its transfer to cheese and ricotta. *J. Dairy Sci*. **2005**, *88*, 1311–1319.

(5) Berner, L. A. Roundtable discussion on milkfat, dairy foods and coronary heart disease risk. *J. Nutr*. **1993**, *123*, 1175–1184.

(6) Chilliard, Y.; Ferlay, A.; Mansbridge, M.; Doreau, M. Ruminant milk fat plasticity: nutritional control of saturated, polyunsaturated, *trans* and conjugated fatty acids. *Ann. Zootech*. **2000**, *49*, 181–205.

(7) Cabiddu, A.; Decandia, M.; Addis, M.; Piredda, G.; Pirisi, A.; Molle, G. Managing Mediterranean pastures in order to enhance the level of beneficial fatty acids in sheep milk. *Small Ruminant Res*. **2005**, *59*, 169–180.

(8) Vlaeminck, B.; Fievez, V.; Cabrita, A. R. J.; Fonseca, A. J. M.; Dewhurst, R. J. Factors affecting odd- and branched-chain fatty acids in milk: a review. *Anim. Feed Sci. Technol*. **2006**, *131*, 389–417.

(9) Griinari, J. M.; Baumann, D. E. Biosynthesis of conjugated linoleic acid and its incorporation into meat and milk in ruminant. In *Advances in Conjugated Linoleic Acid Research*; Yurawecz, M. P., Mossova, M. M., Kramer, J. K. G., Pariza, M. W., Nelson, G. J., Eds.; American Oil Chemist's Society: Champaign, IL, 1999; Vol. *1*, pp 180−200.

(10) Palmquist, D. L.; Beaulieu, A. D.; Barbano, D. M. Feed and animal factors influencing milk fat composition. *J. Dairy Sci*. **1993**, *76*, 1753–1771.

(11) Kelly, M. L.; Kolver, E. S.; Bauman, D. E.; Van Amburgh, M. E.; Muller, L. D. Effect of intake of pasture on concentrations of conjugated linoleic acid in milk of lactating cows. *J. Dairy Sci*. **1998**, *81*, 1630–1636.

(12) Schroeder, G. F.; Dealhoy, J. E.; Vidaurreta, I.; Bargo, F.; Gagliostro, G. A.; Muller, L. D. Milk fatty acid composition of cows fed a total mixed ration or pasture plus concentrates replacing corn with fat. *J. Dairy Sci*. **2003**, *86*, 3237–3248.

(13) Kraft, J.; Collomb, M.; Möckel, P.; Sieber, R.; Jahreis, G. Differences in CLA isomer distribution of cow′s milk lipids. *Lipids* **2003**, *38* (6), 657–664.

(14) Khanal, R. C.; Dhiman, T. R.; Boman, R. L. Changes in fatty acid comoposition of milk from lactating dairy cows during transition to and from pasture. *Livest. Sci*. **2008**, *114*, 164–175.

(15) Atti, N.; Roussini, H.; Othomane, M. H. Milk production, milk fatty acid composition and conjugated linoleic acid (CLA) content in dairy ewes raised on feedlot or grazing pasture. *Livest. Sci*. **2006**, *104*, 121–127.

(16) Perojo, A.; Garcia-Rodriguez, A.; Arranz, J.; Oregui, L. Effects of time spent on pasture on milk yield, body reserves, herbage intake and grazing behaviour. *Options Méditerranéennes, A* **2005**, *67*, 275–279.

(17) Barron, L. J. R; Redondo, Y.; Aramburu, M.; Pérez-Elortondo, F. J.; Albisu, M.; Nájera, A. I.; de Renobales, M. Variations in volatile compounds and flavour in Idiazabal cheese manufactured from ewe′s milk in farmhouse and factory. *J. Sci. Food Agric*. **2005**, *85*, 1660–1671.

(18) ISO (International Organization for Standardization). Milk fat: preparation of fatty acid methyl esters; 2002; ISO standard 15884.

(19) Collomb, M.; Bühler, T. Analyse de la composition en acides gras de la graisse de lait. I. Optimisation et validation d′une méthode générale à haute résolution (Analysis of the fatty acid composition of the milk fat. I. Optimisation and validation of a general method adapted to the research). *Mitt. Lebensm. Hyg*. **2000**, *91*, 306–332.

(20) Rickert, R.; Steinhart, H.; Fritsche, J.; Sehat, N.; Yurawecz, M. P.; Mossoba, M. M.; Roach, J. A. G.; Eulitz, K.; Ku, Y.; Kramer, J. K. G. Enhanced resolution of conjugated linoleic acid isomers by tandem-column silver-ion high performance liquid chromatography. *J. High Resolut. Chromatogr*. **1999**, *22* (3), 144–148.

(21) Collomb, M.; Bütikofer, U.; Maurer, J.; Sieber, R. Composition en acides gras du lait de brebis produit à diverses altitudes (Fatty acid composition of ewe′s milk at different altitudes). *Rev. Suisse Agric*. **2006**, *38* (6), 335–339.

(22) Ruiz, R.; Oregui, L. M.; Herrero, M. Comparison of models for describing the lactation curve of latxa sheep and analysis of factors affecting milk yield. *J. Dairy Sci*. **2000**, *83*, 2709–2719.

(23) Ulbricht, T. L. V.; Southgate, D. A. T. Coronary heart disease: seven dietary factors. *Lancet*. **1991**, *338*, 985–992.

(24) Doreau, M.; Ferlay, A. Digestion and utilisation of fatty acids by ruminants. *Anim. Feed Sci. Tech*. **1994**, *45*, 379–396.

(25) Kucuk, O.; Hess, B. W.; Ludden, P. A.; Rule, D. C. Effect of forage: concentrate ratio on ruminal digestion and duodenal flow of fatty acids in ewes. *J. Anim. Sci*. **2001**, *79*, 2233–2240.

(26) Wilde, P. F.; Dawson, R. M. The biohydrogenation of α-linolenic acid and oleic acid by rumen microorganisms. *Biochem. J*. **1966**, *98*, 469–475.

(27) Rego, A. O.; Rosa, H. J. D.; Regalo, S. M.; Alves, S. P.; Alfaia, C. M. M.; Prates, J. A. M.; Vouzela, C. M.; Bessa, R. J. B. Seasonal changes of CLA isomers and other fatty acids of milk fat from grazing dairy herds in the Azores. *J. Sci. Food Agric*. **2008**, *88*, 1855–1859.

(28) Collomb, M.; Bisig, W.; Bütikofer, U.; Sieber, R.; Bregy, M.; Etter, L. Fatty acid composition of mountain milk from Switzerland: comparison of organic and integrated farming systems. *Int. Dairy J*. **2008**, *18*, 976–982.

(29) Kim, E. J.; Sanderson, R.; Dhanoa, M. S.; Dewhurst, R. J. Fatty acid profiles associated with microbial colonization of freshly ingested grass and rumen biohydrogenation. *J. Dairy Sci*. **2005**, *88*, 3220–3230.
(30) Talpur, F. N.; Bhanger, M. I.; Khooharo, A. A.; Zuhra Memon, G. Seasonal variation in fatty acid composition of milk from ruminants reared under the traditional feeding system of Sindh, Pakistan. *Livest. Sci*. **2008**, *118*, 166–172.
(31) Williams, C. M. Dietary fatty acids and human health. *Ann. Zootech*. **2000**, *49*, 165–180.
(32) Martin, R. E.; Wickham, J. Q.; Om, A. S.; Sanders, J.; Ceballos, N. Uptake and incorporation of docosahexanoic acid (DHA) into neuronal cell body and neurite/nerve growth cone lipids: evidence of compartamental DHA metabolism in nerve growth factor-differentiated PC12 cells. *Neurochem. Res*. **2000**, *25*, 715–723.
(33) Goudjil, H.; Fontecha, J.; Luna, P.; de la Fuente, M. A.; Alonso, L.; Juárez, M. Quantitative characterization of unsaturated and trans fatty acids in ewe′s milk fat. *Lait* **2004**, *84*, 473–482.
(34) Chardigny, J. M.; Destaillats, F.; Malpuech-Brugère, C.; Moulin, J.; Bauman, D. E.; Lock, A. L.; Barbano, D. M.; Mensink, R. P.; Bezelgues, J. B.; Chaumont, P.; Combe, N.; Cristiani, I.; Joffre, F.; German, J. B.; Dionisti, F.; Boirie, Y.; Sébédio, J. L. Do *trans* fatty acids from industrially produced sources and from natural sources have the same effect on cardiovascular disease risk factors in healthy subjects? Results of the *trans* Fatty Acids Collaboration (TRANSFACT) study. *Am. J. Clin. Nutr*. **2008**, *87*, 558–566.
(35) Parodi, P. W. Nutritional significance of milk lipids. In *Advanced Dairy Chemistry*; Fox, P. F., McSweeney, P. L. H., Eds.; Springer: New York, 2006; Vol. *2*, pp 601−639.
(36) Kuhnt, K.; Kraft, J.; Moeckel, P.; Jahreis, G. *trans*-11-18:1 is effectively Δ9-desaturated compared with *trans*-12-18:1 in humans. *Br. J. Nutr*. **2006**, *95*, 752–761.
(37) Walther, B.; Schmid, A.; Sieber, R.; Wehrmüller, K. Cheese in nutrition and health. *Dairy Sci. Technol*. **2008**, *88*, 389–405.

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