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Short Communication

Seasonal changes of CLA isomers and other fatty acids of milk fat from grazing dairy herds in the Azores

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Abstract

BACKGROUND: Season of the year associated with dietary changes has been recognized as a factor implicated in milk fat fatty acid (FA) profile in dairy cows. However, a lack of information exists concerning cows grazing all year round as is practiced in the Azores, where cows are supplemented in winter with maize silage plus concentrates, while in spring the higher grass allowance only requires supplementation with concentrate. The main objective of this study was to detect any seasonal variation of FA profile of milk fat from milk sampled in bulk tanks of 12 Azorean dairy herds.

RESULTS: Compared to winter milk, milk fat from spring presented a higher proportion of CLA *cis*-9,*trans*-11 (14.3 *versus* 9.6 g kg^{-1} FA), C18:1 *trans*-11 (32 *versus* 22 g kg^{-1} FA), C18:2 *trans*-11,*cis*-15 (3.7 *versus* 2.2 g kg^{-1} FA), CLA *trans*-11,*cis*-13 (0.34 *versus* 0.23 g kg^{-1} FA) and C18:3 n-3 (5.7 *versus* 5.4 g kg^{-1} FA). The C18:2 n-6/C18:3 n-3 ratio was lower ($P < 0.05$) in spring. Branched-chain FA, except the *anteiso*-C15:0, were higher in spring, while odd-chain FA (C15:0) were higher in winter.

CONCLUSION: Dairy herd management in the Azores presents a seasonal variation of milk fat FA composition, where the spring milk may present increased potential benefits for human consumers.

Keywords: dairy farms; pasture; season; milk fatty acids; CLA isomers

INTRODUCTION

The Azorean archipelago, located in the Atlantic Ocean, is an important dairy region of Portugal, being responsible for 27% of total milk production of the country. The temperate Atlantic climate presents excellent conditions for the establishment of improved pastures allowing milk production to be heavily based on grazing almost all year round. Nevertheless, as in other regions of the world, grazing dairy cows are commonly supplemented with maize or grass silage, mainly in periods of pasture shortage.

The milk fat produced by cows grazing pasture in spring and summer has long been known to be richer in unsaturated fatty acids (FA), including *trans*-octadecenoic acids and conjugated isomers of linoleic acid (CLA), than milk from confined cows fed on winter diets, based on conserved forages and concentrate.[1] The pasture is a rich source of polyunsaturated fatty acids (PUFA), mainly linolenic acid (C18:3 n-3), which contribute as precursors of C18:1 *trans*-11,[2] which is further desaturated to rumenic acid (C18:2 *cis*-9,*trans*-11, hereafter CLA cis-9,*trans*-11) in animal tissues.[3] Even in conditions where cows are allowed to graze all year round, seasonal modifications in milk fat FA profile are expected to occur associated with changes in feeding strategies.

The main objective of this study was to evaluate the effect of season of the year associated with changes in diet on FA composition, including detailed CLA isomeric profile, of bulk milk samples from dairy farms in the Azores.

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MATERIALS AND METHODS

Twelve Holstein dairy farms were selected based on similar general feeding management with emphasis on winter and spring diets. The diet of all farms selected consisted of pasture plus maize silage plus concentrate during the winter and pasture plus concentrate during the spring. The estimated dry matter (DM) intake (based on farmers enquires) ranged between 4–7 and 5–8 kg cow/day for maize silage and concentrate, respectively. Mean milk production per cow among farms ranged from 5300 to 7000 kg. The herd number ranged from 28 to 75.

In each herd a composite sample was made from four milk samplings of bulk tanks in the two seasons. Samplings were taken on a weekly basis in January and May, corresponding, in the Azores, to representative peaks of winter and spring, respectively. Fat content of bulk-tank milk samples were routinely analysed by automated infrared analysis (Milkoscan 605, Foss Electric, Hillerod, Denmark). The milk fat layer was isolated by centrifugation for 15 min at $822 \times g$ and thereafter lyophilized and stored at $-20\,^{\circ}C$ until analysis for FA composition. FA methyl esters were prepared by direct transesterification of milk fat.[4] Briefly, 50 mg of dry solids was dissolved in 1 mL of *n*-hexane, and after addition of 0.2 mL of potassium hydroxide (2 mol L^{-1} in methanol) the solution was shaken vigorously for 3 min and left to stand for 1 h. After addition of 100 mg of sodium sulfate the solution was mixed again and centrifuged for 5 min and the supernatant was transferred to gas chromatography (GC) vials. FA methyl esters were analysed using an HP6890A gas chromatograph (Hewlett-Packard, Avondale, PA, USA), equipped with a flame ionization detector (GC-FID) and a fused-silica capillary column (CP-Sil 88; 100 m; 0.25 mm i.d.; 0.20 mm film thickness; Chrompack, Varian Inc., Walnut Creek, CA, USA). The column temperature of 100 °C was held for 15 min, increased to 150 °C at a rate of 10 °C min^{-1} and held for 5 min, then increased to 158 °C at 1 °C min^{-1} and held for 30 min, and finally increased to 200 °C at a rate of 1 °C min^{-1} and maintained for 65 min. Helium was used as carrier gas, and the injector and detector temperatures were 250 and 280 °C, respectively. Identification was accomplished by comparison of sample peak retention times with those of FA methyl ester standard mixtures (Sigma, St Louis, MO, USA) and when no commercial standards were available (like C18:1 *trans*-10 and C18:2 *trans*-11,*cis*-15) by using published chromatograms obtained with similar analytic conditions.[5–7]

The CLA methyl ester isomers were separated by Ag^{+}-high-performance liquid chromatography (Ag^{+}-HPLC) using three silver ion columns in series (Chrom-Spher 5 Lipids, 250 mm × 4.6 mm i.d., 5 μm particle size, Chrompack, Bridgewater, NJ, USA), on an HPLC system (Agilent 1100 Series, Agilent Technologies Inc, Palo Alto, CA, USA) equipped with an autosampler and a diode array detector (DAD) adjusted to 233 nm. The mobile phase was *n*-hexane, containing 0.1% acetonitrile and 0.5% diethyl ether, and the flow rate was maintained at 1mL min^{-1}. Standards of CLA isomers (*cis*-9,*trans*-11; *trans*-10,*cis*-12; *cis*-9,*cis*-11 and *trans*-9,*trans*-11) as methyl esters were purchased from Matreya Inc. (Pleasant Gap, PA, USA). The identification of the individual CLA isomers was achieved by comparison of their retention times with those of commercial standards and with values published in the literature.[5,7] In addition, the identity of each isomer was controlled by the typical UV spectra of CLA isomers from the DAD in the range 190–360 nm, using the spectral analysis of Agilent Chemstation for LC 3D Systems Rev. A.09.01. FA composition was expressed as g kg^{-1} of total FA composition using theoretical response factors[8] for FA with chain length lower than 14. The amounts of CLA isomers were calculated from their Ag^{+}-HPLC areas relative to the area of the main isomer CLA *cis*-9,*trans*-11 identified by GC (which comprise both *trans*-7,*cis*-9 and *trans*-8,*cis*-10 CLA isomers) as described by Kraft *et al.*[9] Under our Ag^{+}-HPLC chromatographic conditions the minor C18:2 *trans*-8,*cis*-10 isomer coeluted under the major C18:2 *cis*-9,*trans*-11 peak.

The data were subjected to analysis of variance using the GLM procedure of SAS[10] considering the effect of farm (F) and the effect of season (S) as fixed effects. The data were presented as least square means followed by standard error of the mean (SEM) and probability values (P).

RESULTS AND DISCUSSION

Bulk-tank milk sampled in spring had lower ($P < 0.002$) fat concentration than milk sampled in winter (35.6 *versus* 37.1 g kg^{-1}; 0.295 standard error of the mean). The seasonal effects on general FA profile and detailed C18 FA profile of milk from surveyed farms are presented in Tables 1 and 2, respectively. The milk fat from spring feeding management presented significantly higher proportions in C10:0, C12:0, C14:0, C15:0 and most of the branched-chain FA and lower in C16:0 and C16:1 *cis*-9 than the winter milk.[11]

Considering the C18 fatty acids (Table 2), the spring milk had a higher proportion of C18:1 *trans*-11, C18:2 *trans*-11,*cis*-15, C18:3 n-3, CLA *trans*-11,*trans*-13, CLA *trans*-9,*trans*-11, CLA *trans*-11,*cis*-13, CLA *cis*-11,*trans*-13, CLA *trans*-10,*cis*-12 and CLA *cis*-9,*trans*-11 and a lower proportion of C18:1 *trans*-6/8, C18:1 *trans*-9, C18:1 *cis*-9, C18:1 *cis*-12, C18:1 *cis*-13 and CLA*cis*-9,*cis*-11.

The CLA *cis*-9,*trans*-11 increased 50% from winter to spring, which is a substantial increment considering that the cows on all farms grazed in the two seasons. Nevertheless, the C18:3 n-3 increases only 6% from winter to spring. This modest increase in C18:3 n-3 is probably due to its extensive rumen biohydrogenation, because most of intermediates of

Table 1. The effect of season on the fatty acid profile of milk fat of 12 dairy farms (g kg^{-1} FA)

	Spring	Winter	SEM	$P <$
C4:0	16.3	16.4	0.773	0.976
C6:0	15.3	14.7	0.523	0.466
C8:0	11.1	10.1	0.417	0.116
C10:0	26.8	22.9	1.217	0.044
C11:0	2.18	2.37	0.133	0.337
C12:0	33.4	28.1	1.44	0.026
iso-C14:0	1.09	0.87	0.028	0.002
C14:0	106	98	2.76	0.046
iso-C15:0	6.35	4.44	0.129	0.0001
anteiso-C15:0	9.40	9.27	0.408	0.834
C15:0	12.1	9.64	0.311	0.0001
iso-C16:0	0.07	0.16	0.057	0.258
C16:0	258	273	5.08	0.050
C16:1 *trans*-9	1.60	1.01	0.087	0.002
iso-C17:0	6.86	6.66	0.161	0.417
C16:1 *cis*-9	12.7	15.0	0.367	0.001
anteiso-C17:0	5.50	4.48	0.208	0.005
C17:0	5.99	5.38	0.199	0.055
C17:1 *cis*-9	2.96	2.98	0.129	0.889
Total C18 FA	441	452	11.51	0.509
C20:0	1.40	1.38	0.056	0.836
Others	24.3	21.4	1.03	0.075

Table 2. The effect of season on the C18 fatty acids of milk fat of 12 dairy farms (g kg^{-1} FA)

	Spring	Winter	SEM	$P <$
C18:0	112	115	5.52	0.711
C18:1 *trans*-6 + *trans*-7 + *trans*-8	2.85	3.44	0.184	0.045
C18:1 *trans*-9	2.23	2.68	0.141	0.046
C18:1 *trans*-10	6.42	7.73	1.093	0.414
C18:1 *trans*-11	32.0	21.6	1.68	0.001
C18:1 *cis*-9	208	228	6.72	0.053
C18:1 *cis*-11	11.1	11.8	0.314	0.137
C18:1 *cis*-12	2.69	3.45	0.145	0.004
C18:1 *cis*-13	1.16	1.48	0.066	0.006
C18:1 *trans*-16	6.26	5.92	0.186	0.232
C18:1 *cis*-15	2.22	2.63	0.166	0.105
C18:2 *tt, ct, tc*[a]	9.80	9.25	0.381	0.328
C18:2 *trans*-11, *cis*-15	3.72	2.24	0.215	0.001
C18:2 n-6	17.1	19.2	1.174	0.225
C18:3 n-3	5.72	5.38	0.251	0.003
Total CLA	16.4	11.3	0.614	<0.001
CLA isomers				
trans-12,*trans*-14	0.208	0.175	0.0124	0.092
trans-11,*trans*-13	0.538	0.304	0.0570	0.015
trans-10,*trans*-12	0.089	0.081	0.0035	0.099
trans-9,*trans*-11	0.138	0.104	0.0077	0.010
trans-8,*trans*-10	0.027	0.027	0.0023	0.941
trans-7,*trans*-9	0.045	0.040	0.0020	0.116
trans-6,*trans*-8	0.008	0.012	0.0016	0.094
cis/trans-12,14	0.070	0.067	0.0033	0.640
trans-11,*cis*-13	0.340	0.231	0.0292	0.023
cis-11,*trans*-13	0.006	0.001	0.0006	<0.001
trans-10,*cis*-12	0.009	0.001	0.0021	0.020
cis-9,*trans*-11	14.26	9.61	0.522	<0.001
trans-7,*cis*-9	0.611	0.659	0.0324	0.314
cis-9,*cis*-11	0.012	0.022	0.0026	0.016

SEM, Standard error of the mean.
[a] Sum of several *trans,trans, cis,trans* and *trans,cis* octadecadienoic isomers eluting after 18:1 *cis*-15 and before 18:2 *trans*-11,*cis*-15.

C18:3 n-3 biohydrogenation are increased in spring milk: C18:1 *trans*-11 (+48%), C18:2 *trans*-11,*cis*-15 (+66%), CLA *trans*-11,*trans*-13 (+77%) and CLA *trans*-11,*cis*-13 (+47%). The C18:2 *trans*-11,*cis*-15 is the major octadecadienoic intermediate derived directly from rumen biohydrogenation of linolenic acid.[2] Elgersma *et al*.[12] reported that C18:2 *trans*-11,*cis*-15 ranged between 5.0 and 8.0 mg g^{-1} FA in milk fat of grazing dairy cows, whereas on winter indoor diets it never exceeded 1 mg g^{-1} FA. The values of C18:2 *trans*-11,*cis*-15 in Azorean milk, reported here, are intermediate between the two situations reviewed by Elgersma *et al*.[12] The CLA isomers *trans*-11,*cis*-13 and *trans*-11,*trans*-13 are also intermediates of linolenic acid biohydrogenation[2,13] and its proportion is also increased in spring milk.

Various authors[14–17] found substantial seasonal differences in CLA *cis*-9,*trans*-11 in milk fat, being consistently higher in the spring–summer period, which coincides with the grazing season, as opposed to the winter when cows' diet was based on conserved forages plus concentrates. Based on the comprehensive review of Elgersma *et al*.,[12] the CLA *cis*-9,*trans*-11 proportion of cow's milk fat from EU countries, USA and Canada ranged from 3.4 to 14.1 mg g^{-1} FA. Only milk from Ireland (14.0 mg g^{-1} FA) and Swiss highlands (15.0–21.0 mg g^{-1} FA) presented higher CLA *cis*-9,*trans*-11 than the Azorean milk reported here. For a similar milk production system (all year round pasture – New Zealand), MacGibbon *et al*.[15] reported values of the same magnitude (11.0–14.0 mg g^{-1} FA depending on the season). In this context, the values of CLA *cis*-9,*trans*-11 proportions in fat of milk from the Azores rank high in the world panorama. In this study, the CLA *cis*-9,*trans*-11 proportion among farms ranged from 5.8 to 14.4 mg g^{-1} FA in winter and from 11.6 to 16.8 mg g^{-1} FA in spring. The larger amplitude of proportions of CLA *cis*-9,*trans*-11 (and also of C18:1 *trans*-10 and C18:2 n-6) observed in winter is most probably due to a greater diversity of feeding management among the farms during this period. The CLA *cis*-9,*trans*-11 proportion found in the current study is consistent with those present in Azorean commercial ultrapasturized bovine milk (11.6–14.6 g.kg^{-1}) recently reported by Leite *et al*.[18] As previously reported,[19] the C18:2 *cis*-9,*trans*-11 and C18:1 *trans*-11 in milk fat of farms in the current study were highly related ($r^2 = 0.85$, $n = 24$), which is consistent with the close metabolic interactions existing between both FA.

Odd- and branched-chain FA (OBCFA) in milk fat of ruminants are largely derived from bacteria leaving the rumen. There is an increasing interest in OBCFA as potential diagnostic tools of rumen function (fermentation pattern, bacterial N), anticarcinogenic effects on cancer cells, influence on milk fat melting

point and indicators of ruminant product intake by humans and its relationship with certain disease outcomes.[20] Spring milk exclusively produced from pasture plus concentrate presented a higher proportion ($P < 0.05$) of total branched-chain (29.3 *versus* 25.9 mg g^{-1}, $P < 0.001$) and odd straight-chain (20.3 *versus* 17.4 mg g^{-1}, $P < 0.001$) FA than winter milk produced from pasture plus maize silage. Milk from spring was more concentrated in *iso*-C14:0, *iso*-C15:0 e *anteiso*-C17:0 but did not differ from winter milk in *anteiso*-C15:0, *iso*-C16:0 e *iso*-C17:0. In winter, the milk presented a higher proportion of C15:0. These data suggest that inclusion of maize silage in the diet modifies the microbial ecosystem, favouring the abundance of starch-fermenting bacteria, as discussed by Cabrita *et al*.[21]

Spring milk had a tendency to a higher total PUFA proportion (53.7 *versus* 47.5 mg g^{-1} FA, $P = 0.06$), although most of them are *trans* FA, so the sum of essential PUFA (C18:2 n-6 + C18:3 n-3 is 24.2 mg g^{-1} FA) did not change between season ($P = 0.675$). Spring milk presented a lower C18:2 n-6/C18:3 n-3 ratio (2.59 *versus* 3.75, $P < 0.001$), reflecting the higher ingestion of 18:3 n-3 associated with higher pasture availability. These data, coupled with higher rumenic acid, suggest that spring milk had a better FA profile with regard to consumer health. However, spring milk had a higher ($P = 0.01$) proportion of total *trans* FA (81.3 *versus* 65.3 mg g^{-1} FA). *Trans* FA are generally considered deleterious to consumers' health and the present nutritional guidelines from the World Health Organization recommend that their intake should be lower than 1% of total caloric intake.[22] However, C18:1 *trans*-11 and CLA cis-9,*trans*-11 contribute to 57% of the total *trans* FA in spring milk. Twenty percent of C18:1 *trans*-11 can be converted to CLA cis-9,*trans*-11 in humans.[23] Moreover, recent studies suggest that butters rich in C18:1 *trans*-11 (and CLA cis-9,*trans*-11) have neutral effects on plasma lipids and atherosclerosis induction in rabbits, whereas butters enriched in C18:1 *trans*-10 showed a negative effect.[24]

CONCLUSIONS

This study indicates that the FA composition of milk produced in dairy herds with all year round grazing feeding management differs between spring and winter. In spring, when availability of pasture is higher, as compared to winter when grazing cows must be supplemented with maize silages, milk presents increased rumenic and linolenic acids and decreased palmitic acid, which may have potential benefits for human consumers.

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