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A survey on mono-polyunsaturated fatty acids, desaturase indices and atherogenic index in the milk fat of local breeds (cabannina, varzese and valdostana) reared in northern Italy

Abstract

In this paper the characteristics of the fatty acid profile in local Italian bovine breeds (Cabannina, Varzese and Valdostana) are compared with those of Friesian, a known cosmopolite breed during the first period of lactation. The local breeds show a general trend to have higher unsaturated fatty acid percentages, as well as lower desaturase indices (related to C14, C16 and C18) and atherogenic index, with respect to Friesian cows. The results can add further information aiming to re-evaluate an almost lost local treasure in Northern Italy.

Keywords: desaturase, cabannina, fatty acids, varzese, valdostana

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Faustini M, Chiesa LM, Munari E, Curone G, Colombani C, Vigo D

Department of Veterinary Sciences and Public Health, University of Milan, Italy

Correspondence: Massimo Faustini, Department of Veterinary Sciences and Public Health, University of Milan, Via Celoria, 10, 20134 Milan, Italy, Tel +3902 5031 7939, Email massimo.faustini@unimi.it

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Abbreviations: UFA, unsaturated fatty acids; MUFA, mono unsaturated fatty acids; FA, fatty acids; SCD, Δ^9-stearoyl coa-desaturase; PUFA, polyunsaturated fatty acids

Introduction

In the past decades, milk has been considered a mere supplier of nutrients: although its importance was considered paramount for the development and growth of newborns, a number of aspects regarding the biological functions of milk were still unknown. Several positive functional properties of milk derive from fatty acids (FA), mainly unsaturated fatty acids (UFA), either monounsaturated (MUFA) or polyunsaturated (PUFA) fatty acids.[1] The importance of such acids in human nutrition is evidenced in a huge background of documentation, as reported by FAO on fat and fatty acids in human nutrition report.[2] In particular, UFAs are considered functional components of food because of their positive effects on disease prevention. The ω-6 and the ω-3 fatty acids have demonstrated potential health benefits,[3] by reducing the risk of cardiovascular disease,[4,5] type-2 diabetes, hypertension,[6–8] cancer[9,10] and certain neurological disfunctions,[11,12] Some of them have an antimicrobial function, as showed by Clement et al.,[13] the arachidonic acid (20:4n-6), linoleic acid (18:2n-6) inhibit the germination of *Candidaalbicans in vitro*. In another study[14] the lauroleic acid (C12:1), 11-methyldodecanoic acid (iso-C13:0), myristoleic acid (C14:1n-5), and g-linolenic acid (C18:3n-6) showed antifungal activities against *Aspergillus fumigates* as well as *C. Albicans*.

The vaccine milk contains about 25% of oleic acid (C18:1 *cis*-9) on the total of FA, but also appreciable percentages of myristoleic (C14:1*cis*-9), palmitoleic (C16:1 *cis*-9) acids, and conjugates of linoleic acid (CLA). The absorption of UFA in the ruminal environment is limited by the biohydrogenation process, operated by rumen bacteria.[15] The presence of several UFAs in milk is due mainly to the presence in mammary gland of the enzyme Δ^9-stearoyl CoA-desaturase (SCD): for example, oleic acid derives from stearic acid, while myristoleic acid is synthesized from C14 (myristic acid), and palmitoleic acid from palmitic acid (C16). The activity of SCD accounts for the synthesis of other FA,

The SCD activity can be indirectly determined by the calculation of simple desaturase indices calculated on the base of the substrate: product ratios or analogue calculations.[16] Desaturase indices vary noteworthy between and within subjects, with a heritability of 0.2-0.3.[17,18]

SCD exerts its activity in a non-equal manner: the preferred substrates are C16 and C18 fatty acids;[19] the preferred desaturase index is the C14 index ($\Delta^9$14), since almost all C14:1 cis-9 acid is synthesized by the mammary gland. $\Delta^9$14 correlates positively with SCD activity in milk somatic cells.[20]

Italy, is particularly rich in biodiversity, especially is reported a high number of autochthonous cattle breeds. Three northern breeds, namely Cabannina, Varzese and Valdostana have been appreciated for their milk and characteristics, especially for the rusticity, frugality, fertility and longevity.[21,22] These features make these animals the first choice in marginal areas like mountain, wood and foothill grazes. The great rusticity of these breeds is also noticeable in its low susceptibility to the metabolic or inflammatory disorders/diseases.[23] The autochthonous breeds are better adapted to the local environment, climate, feed and pathogens compared to the cosmopolitan breeds. These breeds have unique and peculiar features resulting from the interaction of its genetic background and the environmental conditions where they live. Unfortunately, the animal husbandry of the 21st century has brought a decline in biodiversity of bovine breeds, due to the abandonment of autochthonous cows in favor of more productive cosmopolitan breeds (Holstein, Brown Swiss and Jersey) only following the milk production increase goal. So these breeds –at least Cabannina and Varzese- are endangered. Nowadays, Cabannina

breed accounts for about 120 brood-females, and Varzese breed for 210 brood-females.[24] Valdostana breed accounts for about 13000 brood-females.[25]

Materials and methods

Animals

The research took place in a herd located in Northern Italy (province of Pavia): a total number of 129multiparous post partum cows with eutocic delivery have been enrolled. The cows were of four different breeds: Friesian (n=30), Cabannina (n=30), Varzese (n=30) and Valdostana (n=39) and were milked twice a day. All subjects were in good health status, as verified by periodic veterinary observations. All cows were fed with diet composed of poliphyte hay and integration of concentrates (flour of cereals and *Leguminosae* (*Fabaceae*), soy-free) Animals were chosen in order to have three classes of lactation stage: milk collections were carried out starting from 40±10days (group A), 70±10days (group B), and 130±10days (group C). All milk samples were taken during the morning milking into a glass tube, immediately forwarded to the laboratory and frozen at -80°C until analysis.

Milk fat determination and fatty acid profiles

The milk fat content of each sample was determined by the UV spectrophotometrical method proposed by Forcato et al.[26] 30-60µl of milk are added of ethanol and stored for a time ~1h at -20°C in order to precipitate interfering proteins and peptides. The supernatants are read at 208nm wavelength in a UV/Vis spectrophotometer.

Milk fat matter was extracted from the thawed samples by the method described by Bligh & Dyer[27] modified by Manirakiza et al.[28] Briefly, 1mL of each sample are transferred in a 15mL Falcon test tube and 3.75mL of a chloroform: methanol 1:2 solution are added. After 10minutes of vortex processing, 1.25mL of chloroform are added to the mixture, and the sample is vortex-stirred for 1min. Finally, 1.25mL of microfiltered distilled water are added and the resulting suspension is vortex mixed again for 1minute. Samples are centrifuged at 2000rpm for 10minutes at 20°C in order to obtain three phases: the supernatant, clear, composed of fat dissolved in chloroform; the intermediate one, mainly composed of proteins and the lowest density one formed by the aqueous medium.

The chloroform-containing phase was filtered with paper and collected in a previously weighted test tube containing anhydrous sodium sulfate, in order to eliminate possible water residues. One hundred and fifty µl of nonadecanoic acid (C:19) were added to each sample as an internal standard for gas chromatography. The solution was dried under mild nitrogen flow and the solid matter quantified. Samples prepared as descripted above, were stored at -80°C till GC analysis were performed. For this purpose, the sample was dissolved in chloroform:methanol 2:1 solution with a ratio of 1mL for 1mg of extract.

Sample were derivatized as described by Moltó-Puigmartí et al.[29] with some modifications: each sample was incubated with 0.5mL of sodium methoxide at 80°C for 10minutes, then they were cooled at 37°C, prior to a new incubation with 0.5mL of boron trifluoride at 80°C for 3minutes; finally the sample was cooled at room temperature. After the addition of 0.5mL of hexane, samples were shaken for 1 minute, then 0.5mL of NaOH saturated solution and anhydrous Na_2SO_4 were added to obtain 3phases.

The supernatant was collected, dried under mild nitrogen flow and suspended in 1mL of hexane. 0.5µl of this solution were injected in a gas chromatograph Trace GC 2000 Series equipped with a GC column (Teknokroma 100mT plus C max 150/prog 250°C, High Polar, Flow N2 0,5mL/min (0.25 mm x 0,20µmm Film) and a pre-column of 5mT (ID=0.25) deactivated). A programmed temperature run was used: the initial oven temperature was 70°C, isothermal for 2'30". Then, temperature was increased at a rate of 3°C min^{-1} to 240°C. The total analysis time was 95min. Gaschromatographic peaks were identified by comparing the retention times of sample with a 37 methylester fatty acid mix (FAME Mix 37, Supelco) and 9-11 and 10-12 linoleic acid conjugate methyl esters (Matreya).

Fatty acids and desaturase indices

On the total peak fatty acids profile, the percentage of 18 unsaturated/polyunsaturated fatty acids (UFA) have been determined by integration of chromatograms by the software Azur for Windows platform. The following UFA were determined (Table 1).

Table 1 Un saturated fatty acids

C14:1cis (myristoleic)
C16:1cis (palmitoleic)
C18:1trans (elaidic)
C18:1cis (oleic)
C18:2trans (linolelaidic)
C18:2cis (linoleic)
C18:3n6 (γ-linolenic)
C18:3n3 (α-linolenic)
CLA 9-11 (C18:2 cis-9 trans-11, conjugated linoleic acid)
C20:1n9cis (cis-11eicosenoic)
C20:2cis (cis-11,14 eicosadienoic)
C20:3n6cis (cis-11,14-eicosatrienoic)
C22:1n9cis (erucic)
C20:3n3cis (cis-11,14,17-eicosatrienoic)
C20:4n6 (arachidonic)
C22:2 (cis-13,16-docosadienoic)
C20:5n3 (cis-5,8,11,14,17-eicosapentaenoic)

Furthermore, the percentages of C14, C16 and C18 fatty acids were taken into account in order to calculate the desaturase indices. Beside the single fatty acid percentages, the total percentage of unsaturated fatty acids (UFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and the UFA to saturated fatty acid ratio (UFA/SFA) were calculated.

Desaturase indices (Δ^9) were calculated as reported by Schennink et al. from the percentages, and total desaturase index ($\Sigma\Delta^9$) was calculated according to Mele et al. on C14, C16 and C18 fatty acids; briefly, the individual Δ was calculated as $\sum Cx{:}n/\Sigma\ (Cx+Cx{:}n)*100$, where x is the number of carbons of fatty acid, and n is for the number of double bonds. The total desaturase index was calculated as:

$$\Sigma\Delta^{9} = \frac{\left(C14:1 + C16:1 + C18:1\right)}{\left(C14 + C14:1 + C16 + C16:1 + C18 + C18:1\right)} x100$$

Atherogenic index (AI) of milk was calculated as follows:

$$AI = \frac{\left[C\text{üüüüüüü}\left(C\quad\right) + \left(C\quad\right)\right]}{\Sigma\left(MUFA + PUFA\right)}$$

Statistical analysis

Data concerning the percentages of fatty acids, the desaturase and atherogenic indices were analyzed by a mixed model analysis of variance, considering the lactation period as the fixed factor, and the subject as random factor. Differences between breeds were evaluated by the Tukey test for multiple comparisons. Data are resumed by mean±standard deviation. Statistical significance was set for all tests at least with $p \leq 0.05$.

Results

The three groups of cows had a mean milk production of 24.17±6.84kg/d (Friesian), 14.24±4.36kg/d (Cabannina), 11.84±4.59 (Varzese), and 9.33±4.50kg/d (Valdostana); the mean fat content was 3.53±1.01%, 4.05±1.14%, 4.27±0.87%, and 3.63±0.81% for Friesian, Cabannina, Varzese and Valdostana, respectively.

Several variables changes over the breeds, while only a few variables show significant changes between lactation periods. A number of differences between breeds are evidenced, in particular between local and Friesian, with higher percentages of UFA, MUFA, PUFA, and a higher UFA/SFA ratio. Varzese cows account for the higher UFA content (about 29%, Table 2), while PUFA percentages are significantly higher in Cabannina and Valdostana breeds. MUFAs percentage in milk fat is higher in local breeds with respect to Friesian, with no differences between Cabannina, Varzese and Friesian. Among MUFAs, Cabannina cow yield a milk with higher levels of C14:1, C18:2*cis*, C18:3n3, while Varzesemilk fat shows higher percentages of C18:1*cis*. Valdostana breed remarkably yielded the higher percentages of a *-trans* FA, C18:1*trans*, with respect to the other breeds; the same breed had notably levels of C18:3n3, CLA 9-11, C20:1, C20:4, C20:5 (Table 2).

Concerning the Δ^9 indices, the $\Delta^9$14 had the higher values in Cabannina breed (about 6.7, Table 1), whereas the highest $\Delta^9$16 mean value (5.3%) was found in Varzese. No differences have been noted for the $\Delta^9$18. The $\Sigma\Delta^9$ index was, for the three local breeds, significantly lower (mean values 3.3 to 3.9%) when compared to Friesian (mean 4.8%, Table 1). The opposite conclusions could be drawn for the AI, significantly higher in Friesian (mean AI=4.8):Cabannina, Varzese and Valdostana breeds maintain lower levels of AI, without differences between groups (mean AI from 3.3 to 3.9, Table 2).

The influence of lactation period is limited for all the considered parameters: a significant increasing trend is observed for C14:1, C18:3n3, CLA 9-11, C20:3n6, $\Delta^9$14, and $\Delta^9$18. On the other side, negative trends over time are seen for C18 and C22:1.

Table 2 Mean percentages of the fatty acids taken into account for the survey. Different superscripts indicate a p<0.05 difference between breeds (Tukey post-hoc test). Asterisks indicate the statistical significance of the effect (breed/time): *-p<0.05; **-p<0.01; ***-p<0.001, n.s.- not significant; arrows on the "time trend" column mark an ascending or descending trend for the variable (p<0.05)

Fatty acid or Index %	Friesian (n=30)	Cabannina (N=30)	Valdostana (n=39)	Varzese (n=30)	Breed p	Time p	Time trend
C14	15.0±1.5	15.29±1.8	14.9±1.8	14.2±1.4	n.s.	n.s.	-
C14:1	0.9±0.30^{b}	1.09±0.3^{a}	1.0±0.3^{ab}	0.9±0.3a^{b}	**	***	↑
C16	41.7±4.6^{a}	37.5±3.32^{b}	36.0±2.8^{b}	36.5±3.8^{b}	***	n.s.	-
C16:1	1.9±0.8^{ab}	2.0±0.7^{ab}	1.5±0.8^{b}	2.0±0.90^{a}	*	n.s.	-
C18	9.4±2.3	9.4±2.2	10.0±1.4	10.4±2.2	n.s.	***	↓
C18:1t	0.7±0.3^{b}	0.7±0.2^{b}	1.6±0.4^{a}	0.8±0.4^{b}	***	n.s.	-
C18:1c	17.3±4.3^{c}	20.1±3.7^{b}	20.3±3.1^{b}	23.2±3.6^{a}	***	n.s.	-
C18:2t	0.2±0.1	0.2±0.1	0.1±0.3	0.2±0.2	n.s.	n.s.	-
C18:2c	1.70±0.60^{b}	2.5±1.5^{a}	1.7±0.5^{b}	1.80±0.8^{b}	**	n.s.	-
C18:3n6	0.001±0.005	0.001±0.005	0.03±0.1	0.05±0.30	n.s.	n.s.	-
C18:3n3	0.2±0.4^{b}	0.6±0.6^{a}	0.6±0.3^{a}	0.4±0.4^{ab}	**	*	↑
CLA 9-11	0.1±0.2^{c}	0.3±0.2^{ab}	0.4±0.3^{a}	0.2±0.3^{bc}	***	***	↑
C20:1	0.09±0.2^{b}	0.07±0.2^{b}	0.2±0.3^{a}	0.04±0.10^{b}	***	n.s.	-
C20:2	0.01±0.03	0.01±0.02	0.01±0.01	0.01±0.04	n.s.	n.s.	-
C20:3n6	0.02±0.03^{b}	0.04±0.04^{ab}	0.04±0.02^{a}	0.04±0.04^{ab}	*	**	↑
C22:1	0.02±0.03	0.01±0.03	0.01±0.01	0.01±0.02	n.s.	*	↓
C20:3n3	0.01±0.02	0.02±0.04	0.02±0.02	0.03±0.08	n.s.	n.s.	-
C20:4	0.01±0.02^{b}	0.02±0.03^{b}	0.07±0.05^{a}	0.02±0.03^{b}	***	n.s.	-
C22:2	0.002±0.01^{b}	0.003±0.01^{b}	0.01±0.02^{a}	0.001±0.02^{b}	***	n.s.	-

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Table Continued..

Fatty acid or Index %	Friesian (n=30)	Cabannina (N=30)	Valdostana (n=39)	Varzese (n=30)	Breed p	Time p	Time trend
C20:5	0.01±0.02^{b}	0.01±0.01^{b}	0.03±0.02^{a}	0.01±0.01^{b}	***	n.s.	-
UFA	23.3±4.8^{b}	27.8±4.4^{a}	27.9±4.0^{a}	29.4±5.6^{a}	***	n.s.	-
MUFA	21.1±4.6^{b}	24.2±4.2^{a}	24.9±3.7^{a}	28.6±5.4^{a}	***	n.s.	-
PUFA	2.2±1.0^{b}	3.6±1.7^{a}	3.0±0.7^{a}	2.8±1.4^{ab}	***	n.s.	-
UFA/SFA	0.3±0.09^{b}	0.4±0.1^{a}	0.4±0.1^{a}	0.4±0.1^{a}	***	n.s.	-
D914	5.5±2.0^{b}	6.7±1.4^{a}	6.5±1.3^{ab}	6.2±2.3^{ab}	*	***	↑
D916	3.9±1.8^{b}	5.1±1.9^{ab}	4.1±2.1^{ab}	5.3±2.2^{a}	*	n.s.	-
D918	65.0±5.4	68.2±4.4	67.0±2.8	67.0±13.9	n.s.	**	↑
SD9	23.1±4.9^{b}	27.1±4.1^{a}	27.4±3.8^{a}	29.2±6.0^{a}	***	n.s.	-
AI	4.8±1.2^{a}	3.9±0.8^{b}	3.7±0.8^{b}	3.3±0.7^{b}	***	n.s.	-

Discussion

The results here reported shows that cattle biodiversity is an important feature –at least at the fatty acids level- for the maintaining and the preservation of the local breeds. The breeds of autochthonous cattle analyzed in the present work show a number of differences from a cosmopolite breed (i.e. Friesian), with an overall higher percentage of UFA, MUFA, and PUFA. Several fatty acids differ between breeds: in particular, the levels of C14 (myristic acid) in all breeds are similar to that reported by White et al.,[30] for Holstein, whereas the unsaturated analogue C14:1 (myristoleic acid) resulted, with respect to the data reported by White et al.,[30] higher in all of the breeds. The percentage of C16 fatty acid in the examined breeds is higher than the percentages reported by White et al.,[30] for Friesian (31.19÷31.67 %) with higher values in Friesian cow than in the other breeds.

The percentages on the single fatty acids are generally in the ranges reported by Jensen,[31] as for oleic acid (C18:1*cis*), with the exception of Friesian, with mean values below the lower limit. Linoleic acid (C18:2*cis*) is –for all breeds- in the range referred by Jensen,[31] while linolenic acid (C18:3) in Friesian and Varzese resulted lower than the limits reported by Jensen.[31]

Unsaturated long-chain fatty acids (>20C) are overall more represented in local breeds with respect to Friesians, in particular C20:1 (mainly present in Valdostana), C20:3n6 (in Cabannina, Valdostana and Varzese), C20:4 (in Valdostana), C22:2 (in Cabannina), C20:5 (in Valdostana). Desaturase indices are generally higher in local breeds than in Friesian, with the exception of $\Delta^9$18, that did not differ between breeds. $\Delta^9$14 indices are quite lower than that referred by Kay et al.,[32] with significant differences between breeds, since Friesian cows show lower values of the index when compared to the other breeds. The same conclusion can be drawn for $\Delta^9$16, lower in our samples than in those reported by Kay et al.[32]

The $\Delta^9$18 did not change among breeds, although a temporal increasing trend ($p<0.01$) was observed. Concerning the total desaturase index ($\Sigma\Delta^9$) resulted significantly higher in local breeds than in Friesian cows; overall, the evaluated $\Sigma\Delta^9$ is lower than that calculated by Kay et al.[32] The desaturase indices $\Delta^9$14 and $\Delta^9$18 increase significantly with lactation (Table 1). These results partially contrast with the findings of Kay et al.,[32] reporting significant variations for all of the indices. The actual desaturase capacity can be, therefore, a matter of discussion for the direct determination of the activity for this enzyme.

The atherogenic index was significantly lower in local breeds (mainly Varzese) when compared to the Friesian; in this breed, the AI values are comparable to those reported by Nantapo et al.[33]

Conclusion

The results confirm, at least partially, that local breeds produce a different milk with respect to a cosmopolite breed as Friesian, so the biodiversity is not only related with the breed but also with the 'milks'. The fatty acid proportions in local breeds milk show significantly higher percentages of unsaturated acids, either mono- or polyunsaturated; these features reflects on the desaturase indices, with the highest mean values in local breeds than in Friesian, and on Atherogenic index, strongly lower in local breeds. The differences in such characteristics can enhance the value of milk in human health terms, due to deep differences in fatty acid composition. These features can contribute to the re-evaluation of bovine local breeds and their products, in order to recover the continuously reducing heads, and enhance the quality of derived products, with positive effects on health, little breeding farm economy and biodiversity.

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Conflict of interest

Author declares that there is no conflict of interest.

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