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Influence of organic systems on Stearoyl-CoA desaturase gene expression in goat milk☆

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ABSTRACT

The effects of organic system on Stearoyl-CoA desaturase (SCD) gene expression in milk somatic cells was evaluated in 30 pregnant pluriparous goats, divided into two homogeneous groups (O and S). Group O was fed according to EC Regulation 834/2007 and led to pasture while group S was stabled and received alfalfa hay as forage. After kids weaning, milk yield was recorded and individual representative milk samples were analysed for chemical composition and fatty acid profile. The SCD gene expression was studied by extraction of mRNA from milk somatic cells and RT-PCR analysis. The body weight of goats of both groups did not show any significant change during the trial. Average milk yield did not differ statistically between the groups while milk from group O showed a significantly higher fat content than those from group S (65.9 vs. 54.3 g/day, $P < 0.01$). Organic system significantly affected the percentages of c9 C18:1, t11 C18:1, octadecadienoic acid, octadecatrienoic acid, monounsaturated fatty acids as well as the c9 t11 conjugated linoleic acid (CLA) (0.810 vs. 0.542 g/100 g of fat, for groups O and S, respectively, $P < 0.01$) and $\sum$CLA (0.860 vs. 0.580 g/100 g of fat for groups O and S, respectively, $P < 0.01$) concentrations in milk. The Stearoyl-CoA desaturase ratios were not different between treatments. Finally, the SCD gene expression was significantly higher in the somatic cells in milk yielded from the "organic" group (AU: 1.5 ± 0.5 vs. 0.39 ± 0.11 for groups O and S, respectively, $P < 0.01$).

1. Introduction

The organic livestock system is governed by an European Regulation (Council Regulations 834/2007) which prohibits breeding without land, stimulates the access of animals to pasture and indicates that dry matter from roughage has to represent at least 60% of total dry matter intake (50% during the lactation), with the aim to guarantee animal welfare and product quality. In addition, the choice of breeds should take account of their capacity to adapt to local conditions. Animal products from organic livestock system increasingly attract consumers because of health aspects, mainly as concerns fatty acid profile. Several studies demonstrated the beneficial effects of n-3 fatty acids in the prevention of several diseases such as coronary heart disease, hypertension, type 2 diabetes, rheumatoid arthritis (Simopoulos, 1999) while conjugated linoleic acid (CLA) is suggested to have immunomodulating, anticarcinogenic and antiartheriosclerosis properties (Pastuschenko et al., 2000; Whigham et al., 2000). CLA is a group of positional and geometric fatty acid isomers derived from octadecadienoic acid of which milk fat is the richest dietary source (Parodi, 1999). The major isomer of

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CLA is cis-9, trans-11 or rumenic acid, which represents up to 80% of total CLA in the food. Ruminant CLA comes from two sources: (1) rumen biohydrogenation; (2) endogenous synthesis in the mammary gland and in the adipose tissue by the activity of Stearoyl-CoA desaturase (SCD) on trans11 C18:1 (TVA, transvaccenic acid), intermediate product of several polyunsaturated fatty acids (PUFA) biohydrogenation (Griinari et al., 2000). Stearoyl-CoA desaturase (SCD) is also the rate-limiting enzyme in the biosynthesis of monounsaturated fatty acids (MUFAs) by the introduction of a cis double bond between carbons 9 and 10 in a spectrum of saturated fatty acids, with preference for C16:0 and C18:0. The expression of SCD is known to change according to animal species, tissue, dietary conditions and environmental factors such as age (Martin et al., 1999), insulin (Daniel et al., 2004) and CLA (Choi et al., 2000). Some PUFA are thought to inhibit SCD by down-regulating its gene expression (Yang et al., 1999). Many animal species are known to have multiple SCD isoforms. To date, four isoforms (SCD1, SCD2, SCD3, and SCD4) have been described in mice (Kaestner et al., 1989; Miyazaki et al., 2003; Ntambi et al., 1988; Zheng et al., 2001), and two isoforms (SCD1 and SCD5) have been described in humans (Wang et al., 2005, Zhang et al., 1999). The presence of different isoforms between species is unclear, while there is evidence for their divergent tissue-specific expression. In ruminants, the SCD1 isoform has been characterised in goats (Bernard et al., 2001), sheep (Ward et al., 1998), and cattle (Chung et al., 2000) and recently it has been described an SCD5 isoform in cattle (Lengi and Corl, 2007) and sheep (Lengi and Corl, 2008). SCD1 is most highly expressed in adipose tissue and liver, while the recently described SCD5 is highly expressed in brain and pancreas (Lengi and Corl, 2008).

Ruminant mammary SCD1 gene expression has been assessed by examining mRNA abundance in samples of mammary tissue collected postmortem (Beswick and Kennelly, 2000), in tissue samples taken by mammary biopsy (Peterson et al., 2003) and in milk somatic cells (Boutinaud et al., 2002; Murrieta et al., 2006; Feng et al., 2007).

In any event, information on the influence of livestock system on mammary SCD gene expression are limited. The aim of this study was to assess the effects of organic system, which indicates the access of animals to pasture rich in precursors of TVA, on SCD gene expression in somatic cells from milk yielded by an autochthonous goat population called 'Cilentana', bred in Cilento (Salerno province, Southern Italy).

2. Materials and methods

Forty-five days before kidding (stage of pregnancy ascertained by ultrasonography), 30 pluriparous pregnant goats (50 ± 1.5 kg and 50 ± 1.8 kg body weight for groups S and O, respectively) were divided into two equal groups (S, stable and O, organic), homogeneous in parity and milk yield at the previous lactation. All the subjects were fed ad libitum oat hay, and 200–300 and 400 g/head/day of concentrate from conventional (group S) or organic production (group O), 45–30 and 15 days before kidding, respectively.

After kidding, group O had free access to pasture (9.00 am to 4.00 pm), constituted by 60% Leguminosae (*Trifolium alexandrinum*, *Vicia* spp.) and 40% Graminee (*Bromus catharticus*, *Festuca arundinacea*, *Lolium perenne*) while group S was housed in a stable and fed alfalfa hay (1.2 kg as fed per head), chosen in order to guarantee the same protein intake for both groups. Indeed, in a previous trial carried out in the same area (Infascelli et al., 2007), the protein content of pasture was close to 16% dry matter (DM). The kiddings were all twins and occurred up to the first week of February. According to EC Regulation No. 834/2007, the concentrate (supplied after provision of forage for group S and after grazing for group O) was gradually increased up to 700 g/head/day. Indeed, as the average body weight of the goats was about 50 kg, and given that at grazing goats are attributed (Rubino, 1996) a voluntary intake of 3.5% of body weight, 700 g represents 40% of the presumed diet consumed by animals.

From day 0 to 60, milk was only suckled by kids while, from the second half of April, goats were milked twice a day. At 68 ± 2.1 days after kidding, body weight was measured and representative milk samples from the two daily milkings were collected. Milk was analysed for protein, fat and lactose contents by the infrared method using a Milko Scan 133B (Foss Matic, Hillerod, Denmark) standardised for goat milk. In addition, total fat of milk samples was separated using a mixture of hexane/isopropane (3/2, v/v), as described by Hara and Radin (1978). Transmethylation of fatty acids was conducted by a base-catalysed procedure according to Christie (1982), with modifications by Chouinard et al. (1999). Fatty acid methyl esters were quantified using a gas chromatograph (ThermoQuest 8000TOP gas chromatograph, equipped with flame ionisation detector; ThermoElectron Corporation, Rodano, Milan, Italy) equipped with a CP-SIL 88 fused silica capillary column [100 m × 0.25 mm (internal diameter) with 0.2-lm film thickness; Varian, Walnut Creek, CA, USA]. Gas chromatograph conditions were set according to Tudisco et al. (2010). Fatty acid peaks were identified using pure methyl ester external standards (Larodan Fine Chemicals, AB, Limhamnsgardens Malmo, Sweden). Additional standards for CLA isomers were obtained from Larodan. Fatty acids in samples were identified by comparing the retention times of peaks with those of the standard mixture. The SCD activity indexes were calculated by the following ratios: C14:1/C14:0, C16:1/C16:0, C18:1/C18:0.

The expression levels of SCD1 gene were studied by extraction of total RNA from milk somatic cells using the method of Feng et al. (2007), with modifications. Each milk sample (300 mL) was decanted into sterile 50-mL conical tubes (6 tubes per sample) and somatic cells were pelleted by centrifugation at $2700 \times g$ for 10 min at 4 °C. Cream and skim milk layers were removed, and cell pellets were washed twice in 5 mL of ice-cold PBS (pH = 7.2) and centrifuged at $2700 \times g$ at 4 °C for 15 min. The 6 cell pellets from each sample were combined in 4 mL of PBS and centrifuged at $2700 \times g$ at 4 °C for 15 min. All the supernatant was discarded, apart from 200 μL, which was used to resuspend the pellet. This was transferred to a 1.5 mL tube and centrifuged at $6000 \times g$ at 4 °C for 15 min. The upper phase (≈100 μL) was discarded and the somatic cells pellet was resuspended in 1 mL of PBS and stored at −80 °C until used for total RNA extraction.

Total RNA was extracted using RNeasy Mini Kit (Qiagen Inc., Valencia, CA) according to manufacturer's instructions, suspended in 50 μL of sterile water containing 0.1% diethyl pyrocarbonate (DEPC). Total RNA concentration and the 260/280 nm and 260/230 nm absorbance ratios were measured using biophotometer (Eppendorf, Hamburg, Germany). RNA quality was determined on Agilent 2100 Bioanalyzer instrument (Agilent Technologies, Santa Clara, CA, USA), based on microcapillary electrophoresis. With this technology, electropherograms and gel-like images can be visually evaluated and an expert software can generate an RNA Integrity Number (RIN), a user-independent assessment of RNA integrity.

Total RNA (1 μg) was incubated in gDNA Wipeout buffer (Qiagen, GmbH, Hilden, Germany) at 42 °C for 2 min to effectively remove contaminating genomic DNA. Then, first-strand cDNA was reverse transcribed using Quantiscript Reverse transcriptase (Qiagen) according to manufacturer's instructions. Negative controls of cDNA synthesis reactions were conducted in the absence of reverse transcriptase and used as template in PCR to verify the absence of genomic DNA contamination for each sample. The resulting first-strand cDNA was diluted in DEPC water (1:50). Specific gene primers (PRIMM, Milano, Italy; concentration adjusted to 10 pmol/μl) were: SCD as described by Tsiplakou et al. (2009) and Cyclophilin A, as described by Flint et al. (2006).

Real-time PCR was performed on ABI Prism 7300 System (Applied Biosystems, Foster City, USA) using SYBR® Green PCR Master Mix (Applied Biosystems). Reactions were carried out in MicroAmp optical tubes and caps with 200 nM of each specific primer and 2.5 μL of diluted cDNA. Each sample was run in triplicates with a non-template control included. PCR cycling consisted of an initial denaturation step at 95 °C for 10 min followed by 40 cycles of denaturation at 95 °C for 15 s and a combined primer annealing-extension step at 60 °C for 1 min in which fluorescence was

Table 1
Average chemical composition (% dry matter), energy value (UFL/kg dry matter) and fatty acid profile (% of total fatty acids) of feeds.

	Hay	Concentrate		Pasture
		Stable group	Organic group	
Chemical composition				
Crude protein	16.1	18.1	17.3	16.5
Ether extract	1.60	3.10	4.20	2.00
NDF	43.6	27.0	26.0	49.0
ADF	32.5	11.4	10.7	34.0
ADL	5.30	3.00	2.90	4.90
UFL	0.75	1.03	1.10	0.76
Fatty acid profile				
SFA[a]	24.9	23.3	22.9	18.1
MUFA[b]	7.34	17.8	18.2	5.36
PUFA[c]	63.1	59.0	61.2	77.0
C18:2	16.8	48.3	49.1	27.2
C18:3	35.7	9.20	9.84	43.3

[a] Saturated fatty acid.
[b] Monounsaturated fatty acid.
[c] Polyunsaturated fatty acid.

measured. A melting curve was produced after completion of the thermal PCR program to check the presence of one gene-specific peak and the absence of primer dimer. The SCD mRNA expression was calculated by relative threshold cycle numbers (CT) towards Cyclophilin A housekeeping gene following the formula $2^{-(\Delta CTSCD - \Delta CTcyclophilin\ A)}$.

Samples of pasture were collected from three different areas (2.5 m^2 each) at no less than 3 cm from the ground. After weighing, herbage samples were air-oven dried at 65 °C, milled through a 1 mm screen and stored. Samples of pasture, alfalfa hay and concentrate were analysed for chemical composition (AOAC, 2000; Van Soest et al., 1991) and their nutritive value were calculated according to INRA (1978). For the fatty acid profiles of pasture, alfalfa hay and concentrate, the total fat was extracted according to Folch et al. (1957). For trans-methylation and quantification of fatty acids the same procedures indicated for milk samples, were used.

Data were analysed by ANOVA and the comparison among the mean values was performed by using the Tukey test (SAS, 2000).

3. Results

Neither hay or concentrate refusals were registered for group S as well as no concentrate refusals were recorded for group O. Thus, the dry matter intake was similar for both groups. Indeed, as concerns pasture intake, Rubino (1996) reported 20 g/kg body weight (BW) as average pasture DM intake of goats in the inlands of south Italy. In this trial, pasture intake for goats of 50 kg BW was estimated to be equal to 1 kg DM which is similar to the 1.2 kg as fed of hay administered to group S. The average chemical compositions, nutritive value and fatty acid profile of alfalfa hay, concentrates and pasture are reported in Table 1. The ingredients of concentrates are reported in Table 2. The concentrate from organic production showed a slightly higher fat and a lower protein content than that from conventional production, resulting in a similar nutritive value (UFL 1.10 vs. 1.03, for organic and conventional, respectively). The fatty acid profile between the concentrates was also similar. According to our previous results (Infascelli et al., 2007), pasture had 16.5% protein/dry matter (PG/DM) and 0.76 UFL, both equal to the values of alfalfa hay (16.1% PG/DM and 0.75 UFL). Octadecadienoic (C18:2) and octadecatrienoic acids (C18:3) were higher in pasture than in alfalfa hay.

Table 2
Ingredients (% as fed) of concentrates fed by stable or organic group.

	Stable group	Organic group
Soft wheat bran	26.6	26.6
Soybean s.e.	15.1	–
Corn	15.0	15.0
Dried beet pulp	12.0	12.0
Sunflower	10.0	14.5
Faba bean	–	10.6
Corn gluten feed	7.0	7.0
Dried citrus pulp	6.5	6.5
Sugarcane molasses	5.6	5.6
$CaCO_3$	0.5	0.5
Dicalcium phosphate	0.8	0.8
Vitamin-mineral premix	0.2	0.2
NaCl	0.7	0.7

The body weight of goats of both groups did not show any significant change during the trial. Average milk yield did not statistically differ between the groups (g/head/day 1432 vs. 1428, for groups O and S, respectively). Group O showed a significantly higher milk fat production (65.9 vs. 54.3 g/day $P < 0.01$) than group S, while protein and lactose were unaffected by the feeding system. As concerns milk fatty acid profile, group O showed a significantly higher concentration of c9 C18:1, t11 C18:1, octadecadienoic acid, octadecatrienoic acid (C18:3), c9 t11 CLA, total CLA (ΣCLA), MUFA and PUFA, than milk of group S. The ratios c9 C14:1/C14:0, c9 C16:1/C16:0, c9 C18:1/C18:0 were higher in milk from group O respect to group S (Table 3).

The RNA yield extracted from 300 mL milk samples was 4.31 ± 1.4 and 4.32 ± 1.5 for groups O and S, respectively. According to Feng et al. (2007), these values were considered sufficient for the following analysis.

The mean RIN obtained for all extracted samples was 7.0 ± 0.8 and 7.1 ± 0.9 for groups O and S, respectively. This value is higher than 6, the threshold for high and low quality RNA defined by Schroeder et al. (2006).

SCD expression was significantly affected by the feeding system. Indeed, in the somatic cells of milk yielded from the "organic" group, the values, expressed in arbitrary units (AU), relative to Cyclophilin A mRNA, were 1.5 ± 0.5 vs. 0.39 ± 0.11 for groups O and S, respectively ($P < 0.01$) (Fig. 1).

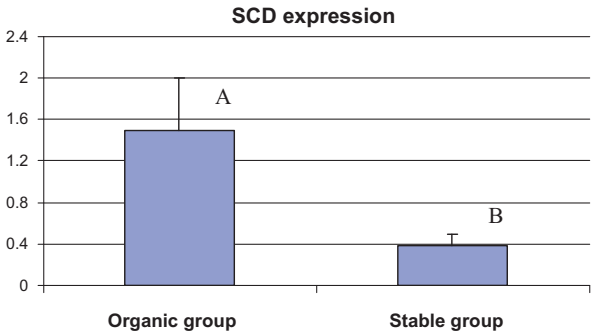

Fig. 1. Relative SCD expression (AU ± SEM) in somatic cells from milk yielded by organic or stable group.

Table 3
Milk yield (g/day/head), chemical composition (g/d) and fatty acid profile (g/100 g of fat) for stable and organic group.

	Stable group	Organic group	SEM[e]
Number of animals	15	15	
Milk yield	1428	1432	36.48
Fat	54.3B	65.9A	1.36
Protein	51.5	51.3	1.63
Lactose	66.2	65.6	1.69
C14:0	9.20	8.66	0.27
c9 C14:1	0.53	0.56	0.02
C16:0	19.32	18.9	0.42
C16:1	1.30	1.31	0.06
C18:0	8.29	7.81	0.38
c9 C18:1	17.1B	19.1A	0.33
t11 C18:1	1.70b	1.83a	0.01
C18:2 n-6	2.07B	2.77A	0.13
C18:3 n-3	0.57B	0.81A	0.03
C20:0	0.24	0.24	0.01
C20:2	0.20	0.21	0.01
C22:0	0.17	0.17	0.01
C20:3	0.50	0.48	0.02
C20:4	0.26	0.25	0.01
c9 t11 CLA[a]	0.542B	0.810A	0.05
t10 c12 CLA[a]	0.024	0.031	0.9×10^{-3}
c9 c11 CLA[a]	0.017	0.020	0.9×10^{-4}
ΣCLA[a]	0.580B	0.860A	0.04
SFA[b]	60.12	58.94	0.62
MUFA[c]	20.83b	23.01a	0.43
PUFA[d]	3.60b	4.52a	0.45
c9 C14:1/C14:0	0.058	0.065	0.002
c9 C16:1/C16:0	0.067	0.069	0.001
c9 C18:1/C18:0	2.06	2.44	0.007

A, B: $P < 0.01$; a, b: $P < 0.05$.
[a] Conjugated linoleic acid.
[b] Saturated fatty acids.
[c] Monounsaturated fatty acids.
[d] Polyunsaturated fatty acids,
[e] Standard error of mean.

4. Discussion

The feeding system did not affect body weight and milk yield. It has to be underlined that the energy requirements were satisfied for both groups. Indeed, according to Rubino (1996), average pasture DM intake of goats in the inlands of south Italy is 20 g/kg body weight (BW) and the energy requirements for maintenance and milk production of local genotype are 0.0365 UFL/kg metabolic weight ($MW = BW^{0.75}$) and 0.41 UFL/kg fat-corrected milk (4% fat), respectively. In this trial, goats of 50 kg BW ingested 1 kg DM of pasture, which is equal to 0.76 UFL, while energy requirements were equal to 1.29 UFL (0.69 UFL for maintenance, plus 0.60 UFL for milk production). The deficit of 0.53 UFL was met by concentrate. In group S, the intake of 1.2 kg fed as alfalfa hay plus concentrate met the energy requirements.

The organic group showed a significantly higher milk fat production. The positive effect of pasture on milk fat has also been reported by Decandia et al. (2007) who, comparing two groups of goats supplemented with the same level of concentrate mixture, found a significantly higher milk fat percentage for the pasture group than that for the group confined to no grazing and fed alfalfa hay.

Concerning milk fatty acid profile, the differences registered between the two groups are due to the pasture. Indeed, milk PUFA content can be increased by using PUFA-rich feedstuffs like oils or oilseeds; however, Griinari and Baumann (1999) pointed out that grazing in pasture is also a good alternative way of increasing the level of milk PUFA, as also confirmed by Banni et al. (1996) in sheep and Dhiman et al. (1999) in dairy cows. In addition, Cabiddu et al. (2004) showed that the overall PUFA content in milk from sheeps grazing pastures rich in legumes was higher (on average 6% of total fatty acids) than that from stall-fed sheeps (4.07% of total fatty acids). In this trial, both octadecadienoic and octadecatrienoic acids were higher (g/100 g fat: 2.77 vs. 2.07 and 0.81 vs. 0.57, respectively; $P < 0.01$) in milk from the organic group, probably due to the higher content of both acids in the pasture compared with that in alfalfa hay (C18:2: 27.2 vs. 16.8% and C18:3: 43.3 vs. 35.7% of total fatty acids). Bergamo et al. (2003), Ellis et al. (2005) and Tsiplakou et al. (2010) also reported significantly higher levels of octadecatrienoic acid in organic milk vs. conventional milk from buffalo, cows and dairy goats, respectively. The organic system affected milk content of c9 t11 CLA and total CLA (ΣCLA), according to Bergamo et al. (2003) in buffalo, Sanz Sampelayo et al. (2007) in goats and Tsiplakou et al. (2010) in sheep. However, these authors did not find differences between organic and conventional feeding system in goats. According to Kelly et al. (1998), the higher concentration of milk CLA found in the organic group could be due to the type and source of dietary carbohydrates, which may influence the rate of microbial fermentation in a way that alters the rate of CLA production or the utilisation by rumen microbes and, therefore, the concentration of CLA in milk fat. Indeed, the higher levels of octadecadienoic and octadecatrienoic acids – the main precursors of c9 t11 CLA – in the pasture can explain the higher CLA content in milk from goats of the organic group (Kemp and Lander, 1984; Kim et al., 2000). Finally, according to Aii et al. (1988), the lower content of c9 t11 CLA in milk of stable goats may also be related to the loss of fatty acids precursor during the hay-making process.

SCD activity can be measured by comparing the product:substrate ratios of certain fatty acids. There are four main products of SCD activity in the mammary gland of ruminants: c9 C14:1, c9 C16:1, c9 C18:1 and CLA, which are produced from C14:0, C16:0, C18:0 and trans11 C18:1, respectively. According to Lock and Garnsworthy (2003), the best indicator of SCD activity is the c9 C14:1/C14:0 ratio because all of the C14:0 in milk fat is produced via de novo synthesis in the mammary gland; consequently, desaturation is the only source of C14. Increasing c9 C14:1/C14:0 ratio values would indicate an increase of SCD activity. In this trial, despite the value was higher in milk from group O, differences were not significant. In the mammary gland of ruminants, SCD1 is known to be responsible for the production of about 80% of c9 t11 CLA, which is secreted in milk (Lock and Garnsworthy, 2003), therefore, part of the increase of CLA could be due to the higher amount of substrate t11 C18:1 for SCD in group O.

SCD expression was higher in the somatic cells of milk yielded from the organic group. The regulation of SCD by dietary factors has been largely investigated in rodents (Ntambi, 1999), while in ruminants the results are conflicting. Indeed, Ahnadi et al. (2002) and Harvatine and

Bauman (2006) found a depression of mammary SCD mRNA abundance when lactating cows were fed protected PUFA, while Bernard et al. (2005, 2009a, 2009b) did not register any significant effect in goats. In the current study, the organic group ingested a higher amount of both C18:2 and C18:3 than the stable group, thus probably resulting in an up-regulation of the SCD expression. According to Bernard et al. (2005), further studies are necessary to elucidate the mechanism underlying this regulatory process as a function of nature and level of dietary lipids.

5. Conclusion

The results of this study showed higher levels of MUFA, octadecadienoic acid, octadecatrienoic acid and CLA in milk from goats bred with the organic system, thus confirming the influence of pasture on the nutritional value of milk. Part of the increase in milk CLA in the organic group could be due to the higher amount of substrate t11 C18:1 for SCD rather to differences in SCD activity as suggested by the absence of differences in the Stearoyl-CoA desaturase ratios between treatments. Finally, the SCD1 gene expression seems to be up-regulated by the higher amount of both C18:2 and C18:3 in the pasture.

Disclosure statement

None of the authors have any financial or personal interest that would inappropriately influence or bias the contents of this paper.

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