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Influence of pasture on goat milk fatty acids and Stearoyl-CoA desaturase expression in milk somatic cells☆

Raffaella Tudisco*, Micaela Grossi, Serena Calabrò, Monica Isabella Cutrignelli, Nadia Musco, Laura Addi, Federico Infascelli

Department of Veterinary Medicine and Animal Science, University of Naples Federico II, via F. Delpino, 1, 80137 Naples, Italy

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ABSTRACT

The effect of pasture fatty acid profile along the year on the Stearoyl-CoA desaturase (SCD) expression was evaluated over 5 months using 30 pluriparous goats, delivered in February. The animals had free access to pasture constituted by 60% Leguminosae and 40% Gramineae, and received 500 g/head/day of concentrate. From the second half of April, goats were milked twice a day for 5 months. Daily milk yield was recorded and, monthly, representative individual milk and pasture samples were analysed for chemical composition and fatty acid profile. The SCD expression was studied by extraction of mRNA from milk somatic cells and analysed by RT-PCR. Average milk yield, fat and protein were 1420 (g/d), 4.45 (%) and 3.62 (%), respectively. Milk yield decreased along the lactation ($P < 0.01$) while milk fat was higher in August. Milk total CLA showed the highest levels ($P < 0.01$) in June and August (mg/100 g fat: 0.98 and 1.21, respectively) as consequence of the highest levels of pasture C18:2 and C18:3 in June (% of total FA: 34.1 and 42.7, respectively) and August (% of total FA: 42.7 and 46.3, respectively). The grazing season as well as lactation stage affected the SCD mRNA abundance determined from milk somatic cells with values (arbitrary units) that progressively decreased from April (1.95) until June (1.40), increased in July (1.70) and decreased again in August (0.83).

1. Introduction

Conjugated linoleic acids (CLAs), suggested to have immunomodulating, anticarcinogenic and antiatherosclerosis properties (Pastuschenko et al., 2000; Whigham et al., 2000) are a group of positional and geometric fatty acid isomers derived from octadecadienoic acid of which the major isomer is cis-9, trans-11 or rumenic acid (up to 80% of total CLA in the food). The presence of CLA in ruminants milk results from the isomerization and biohydrogenation of unsaturated fatty acids [octadecatrienoic (C18:3n-3) and octadecadienoic (C18:2n-6) acids] by rumen bacteria as well as the activity of Stearoyl-CoA desaturase (SCD) in the mammary gland on trans-11 C18:1 (TVA, trans-vaccenic acid), intermediate product of several polyunsaturated fatty acids (PUFA) biohydrogenation. In addition, SCD plays a role in the biosynthesis of mono-unsaturated fatty acids (MUFAs) introducing a cis double bond between carbons 9 and 10 in a spectrum of saturated fatty acids, mainly myristic (14:0), palmitic (16:0), stearic (18:0) and vaccenic acids. Concerning the nutritional impact on SCD gene expression, studies performed in goats revealed that the supplementation of sunflower-seed oil rich in oleic acid (Bernard et al., 2005a), sunflower-seed oil (18:2 rich), or linseed oil (18:3 rich) in

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* Corresponding author. Tel.: +39081292981.
E-mail address: tudisco@unina.it (R. Tudisco).

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diets based either on grass hay (Bernard et al., 2009a) or on maize silage (Bernard et al., 2009b) did not affect mammary SCD mRNA abundance. Similar results have been reported by Bernard et al. (2005b) in lucerne hay-based diets supplemented with soya beans. On the contrary, mammary SCD mRNA decreased supplementing grass hay-based diets with formaldehyde-treated linseed (Bernard et al., 2005a). Recently, Bernard et al. (2012) found that the level and type of starchy concentrate added to diets supplemented with sunflower-seed oil did not affect the mammary SCD gene expression. Similar results have been reported by Zhu et al. (2013) supplementing garlic oil to corn silage based diet.

According to Yang et al. (1999) some PUFA inhibit SCD by down-regulating its gene expression; in addition Lock and Garnsworthy (2003), who found higher CLA concentration in milk of pasture-raised animals than in those fed with dry diets, suggest that it could be due at least in part to an increased SCD activity in the mammary gland in relation with the different fatty acid composition of pasture during the growth stages. Some authors have investigated on the relationship between pasture and milk fatty acid profile, including CLA, and SCD expression, in goats (Tsiplakou et al., 2006; Di Trana et al., 2008; D'Urso et al., 2008; Ataşoğlu et al., 2009; Tudisco et al., 2010, 2012), but information on the influence of pasture fatty acid profile along the year on mammary SCD gene expression are limited. The aim of this study was to assess the effects of grazing season (from April to August), particularly referred to C18:2n-6 and C18:3n-3 levels in the pasture, on milk fatty acid composition, on milk delta-9 desaturation ratios commonly used as indicators of SCD activity and on mammary SCD gene expression of an autochthonous goat population called 'Cilentana', extensively bred in Cilento (Salerno province, southern Italy). Gene expression was studied using milk somatic cells as previously reported in goats (Boutinaud et al., 2002; Modesto et al., 2013), beef cows (Murrieta et al., 2006) and dairy cows (Feng et al., 2007; Boutinaud et al., 2008; Jacobs et al., 2013).

2. Materials and methods

The trial was carried out using 30 goats (3rd parity; 50 ± 2.5 kg body weight), delivered in February, which had free access to pasture (9.00 am to 4.00 pm) constituted by 60% Leguminosae (*Trifolium alexandrinum*, Vicia spp.) and 40% Gramineae (*Bromus catharticus*, *Festuca arundinacea*, *Lolium perenne*) and received 500 g/head/day of concentrate. From day 0 to 60, milk was suckled only by the kids; successively the goats were milked twice a day for 5 months.

2.1. Diet analysis

Samples of pasture were collected day 15 of each month 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 and concentrate were analysed for chemical composition (AOAC, 2000; Van Soest et al., 1991) and their nutritive value calculated according to INRA (1978) (Table 1).

Fatty acid profile of pasture was monthly analysed. Total fat was extracted according to Folch et al. (1957). 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 ionization 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). Fatty acids in samples were identified by comparing the retention times of peaks with those of the standard mixture.

Table 1
Average chemical composition (% dry matter) and energy value (UFL/kg dry matter) of pasture and concentrate.

	Pasture	Concentrate[a]
Crude protein	16.6	18.0
Ether extract	2.00	3.00
NDF	49.3	27.0
ADF	34.1	11.5
ADL	5.00	3.00
UFL	0.76	1.03

NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin.

[a] Ingredients (% as fed): soft wheat bran 26.6; corn gluten feed 7.0; dried citrus pulp 6.5; dried beet pulp 12.0; sunflower 14.5; corn 15.0; faba bean 10.6; sugarcane molasses 5.6; $CaCO_3$ 0.5; dicalcium phosphate 0.8; vitamin–mineral premix 0.2; NaCl 0.7.

2.2. Milk analysis

From April to August, daily milk yield was recorded daily and representative individual milk samples, collected day 15 of each month and obtained by mixing the production of the two daily milking, were analysed for protein, fat and lactose contents by the infrared method using Milko Scan 133B (Foss Matic, Hillerod, Denmark) standardized 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) while for transmethylation and quantification the same procedures indicated for pasture samples were used. Additional standards for CLA isomers were obtained from Larodan. The SCD activity index was calculated from data of fatty acid profile using the ratio C14:1/C14:0 (Lock and Garnsworthy, 2003).

The expression of SCD gene was studied by extraction of total RNA from milk somatic cells using the method of Mura et al. (2013). Briefly, each post-milking sample (300 mL; Boutinaud et al., 2002) was decanted into sterile 50-mL conical tubes (six tubes per sample) and somatic cells were pelleted by centrifugation at 2700 × *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 supplemented with EDTA (ethylene-D-amine-tetra-acetic-acid) 0.5 mM to eliminate casein and fat globules and DEPC (D-ethyl-pyro-carbonate) 0.1% to inactivate the RNAases and other enzymes. After the centrifugation (2700 × *g* at 4 °C for 15 min), six cell pellets from each sample were combined in 4 mL of PBS and again centrifuged at 2700 × *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 × *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