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Fatty Acid Profile and CLA Content of Goat Milk: Influence of Feeding System

Raffaella Tudisco[1], Micaela Grossi[1], Laura Addi[1], Nadia Musco[1], Monica Isabella Cutrignelli[1], Serena Calabrò[1] & Federico Infascelli[1]

[1] Department of Veterinary Medicine and Animal Science, University of Naples Federico II, Italy

Correspondence: Federico Infascelli, Department of Veterinary Medicine and Animal Science, University of Naples Federico II, Italy. Tel: 39-081-253-6074. E-mail: infascel@unina.it

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Abstract

There is great interest in producing bioethanol from biomass and there is much emphasis on exploiting The effect of pasture and of diet linseed supplementation on fatty acid profile of milk was evaluated using 45 pluriparous goats, equally divided after kidding into three homogeneous groups (H, P and L). Group H (control) and L were housed in a stall receiving alfalfa hay, while group P was led to pasture. The groups received concentrate which for group L had linseed as ingredient. Average milk yield did not statistically differ between the groups. Groups P showed significantly higher fat content than group H and L (4.62% *vs* 3.70%, and 3.90% respectively for groups P, H and L; $P < 0.01$). On the contrary, milk from goats of group H and L had significantly higher content of lactose (4.65% and 4.61% *vs* 4.57%, respectively for groups H, L and P; $P < 0.05$). The levels of C18:1 *cis9*, C18:1 *trans11* and MUFA in milk were significantly ($P < 0.05$) increased by both the pasture and linseed, while linoleic acid (C18:2) and PUFA were significantly ($P < 0.05$) higher in group P compared either to H or to L groups. The highest values of CLAs, either as *t11* CLA and *t10 c12* CLA or as ΣCLA, have been registered in milk of group P, the lowest in group H. These parameters in milk of group L were significantly ($P < 0.05$) lower or higher than those of group P and H, respectively.

Keywords: goat, feeding system, fatty acids, CLA

1. Introduction

Goat milk is considered an alternative for consumers who are allergic to cow's milk. As concerns nutritional quality, they differ significantly for lipid composition, goats milk being much higher in butyric (C4:0), caproic (C6:0), caprylic (C8:0), capric (C10:0), lauric (C12:0), myristic (C14:0), palmitic (C16:0), linoleic (C18:2), but lower in stearic (C18:0), and oleic acid (C18:1). Thus, almost 20% of the fatty acids in goat's milk are short chain fatty acids, which are readily digested (Jennes, 1980) while the level of medium chain fatty acids (55%) is relatively high (Boza & Sanz Sampelayo, 1997). Increasing milk polyunsaturated fatty acids (PUFAs) content through animal diet improves milk nutritional value; indeed, they have been associated with a decrease in the risk of heart disease (Albert et al., 1988). In particular, many researches have focussed on the antioxidant and anticarcinogenic properties of a class of the conjugated linoleic acids (CLA) (Parodi, 1999) which come from the rumen biohydrogenation of linoleic and linolenic acids. According to some authors (Nudda et al., 2006; Tudisco et al., 2010; Tudisco et al., 2012) the levels of both these acids in animal diet could affect milk CLA contents. The aim of present trial was to evaluate the effect of pasture and linseed intake on the fatty acid profile and CLA content of milk yielded by an autochthonous goat population called "Cilentana", extensively bred in Cilento (Salerno province, Southern Italy).

2. Materials and Methods

Forty five pregnant, pluriparous goats (50 ± 2.5 kg body weight) were divided into three groups (H, P and L) homogeneous in parity and milk production at the previous lactation. All the animals were fed oat hay *ad libitum* and 200-300 and 400 g/head/d of a concentrate [crude protein (CP) 18% of dry matter (DM); 1.03 Feed Units of Lactation (UFL)/kg DM; 1.4% Ca; 0.7% P], respectively 45 - 30 and 15 days before kidding. Group L received concentrate containing extruded linseed (30% as fed). After kidding, group H (control) and L were housed in a stable, while group P had free access to pasture (9.00 a.m-4.00 p.m.), constituted by 60% leguminosae (*Trifolium alexandrinum*, *Vicia spp.*) and 40% graminee (*Bromus catharticus*, *Festuca arundinacea*, *Lolium perenne*). In previous trials (Infascelli et al., 2005; Tudisco et al., 2010; Tudisco et al., 2012) carried out in the same area, the protein content of pasture was close to 16% dry matter (DM), thus groups H and L received alfalfa hay selected to guarantee the same protein intake for all the groups. The daily intake of alfalfa hay, measured subtracting the refusals to the administered amounts, was 1.2 kg as fed/head. The kiddings were all twins and occurred up to the 1st week of February 2012. Concentrate administration (after forage for group H and L and after grazing for groups P) was gradually increased up to 700 g/head/d. From day 0 to 60 milk was suckled only by the kids. After kid sold (second half of April) goats were milked twice per day for 5 months. Milk yield was recorded monthly and milk samples representative of the two daily milking were analysed for: protein, fat and lactose contents whit infrared method using Milko Scan 133B (Foss Matic, Hillerod, Denmark) standardised for goat milk. In addition, total fat from 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-catalyzed procedure according to Christie (1982) with modifications by Chouinard et al. (1999). Fatty acid methyl esters were quantified using a gas chromatograph (ThermoQuest 8000 ^{TOP} gas chromatograph, equipped with flame ionization detector; ThermoElectron Corporation, Rodano -Milano- Italy) equipped with a CP-SIL 88 fused silica capillary column (100 m × 0.25 mm (i.d.) with 0.2-µm film thickness; Varian, Inc. Walnut Creek, CA). Gas chromatograph conditions were the following: oven temperature was initially 70 °C for 4 minutes, then ramped by 13 °C/min to 175 °C and maintained for 27 min, then ramped to 215 °C by 3 °C/min, maintained for 38 min up to come back to 70 °C at 10 °C/min. Inlet and detector temperatures were 250 °C and and 260 °C, respectively. The split ratio was 100:1. The helium carrier gas flow rate was 1 ml/min, hydrogen flow to the detector was 30 ml/min, airflow was 350 ml/min, and the flow of helium make-up gas was 45 ml/min. Fatty acid peaks were identified using pure methyl ester standards (Larodan Fine Chemicals, AB, Limhamnsgårdens Malmö, Sweden). Additional standards for CLA isomers were obtained from Larodan. Fatty acids in samples were identified by comparing the retention times of peaks with that of standard mixture.

Monthly, samples of pasture were collected by cutting three sample areas (2.5 m^2 each) at 3 cm height above the ground. After weighing, the herbage samples were air-oven dried at 65 °C, milled through a 1mm screen and stored. Samples of pasture, alfalfa hay and concentrates were analysed for chemical composition (AOAC, 2000; Van Soest, Robertson, & Lewis, 1991). The nutritive value was calculated according to INRA (1978). Concerning pasture, alfalfa hay and concentrates fatty acids profile, the total fat was extracted according to Folch, Lees and Sloane (1957) while their transmethylation and quantification followed the scheme described for milk samples.

2.1 Statistical Analysis

The data were analysed by ANOVA using the General Linear Model (GLM) procedure of SAS (2000) including the group effect as a fixed effect and the month of sampling as a repeated measure.

The interaction between effects was evaluated. The comparison among the means was performed with the Tukey test (SAS, 2000).

3. Results

Table 1 shows the average chemical compositions, nutritive value and fatty acid profile of pasture, alfalfa hay and concentrates.

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

	Hay	Concentrate H-P	Concentrate L	Pasture
Chemical composition				
Crude protein	16.3	17.8	17.0	16.3
Ether extract	1.5	3.1	4.0	1.8
NDF	44.2	26.9	25.8	48.2
ADF	31.5	11.7	10.3	35.0
ADL	5.6	2.5	2.8	5.0
UFL	0.75	1.03	1.03	0.76
Fatty acid profile				
SFA	25.2	23.3	25.0	16.9
MUFA	7.02	17.7	14.3	6.2
PUFA	64.3	59.0	60.7	76.9
Linoleic acid	17.1	54.7	42.3	23.9
Linolenic acid	36.8	6.0	16.7	44.5

SFA = saturated fatty acid; MUFA = mono-unsaturated fatty acid; PUFA = poly-unsaturated fatty acid.

The energy requirements of all the groups were satisfied along the experiment. Indeed, according to Rubino (1990) average pasture DM intake of goats in the inlands of South Italy is equal to 20 grams/kg body weight (BW) and the energy requirements for maintenance and milk production of local genotype goats equal to 0.0365 UFL/kg metabolic weight (MW = $BW^{0.75}$) and 0.41 UFL/kg fat corrected milk (4% fat), respectively. In present trial, 50 kg BW goats ingested 1 kg DM at pasture, equal to 0.76 UFL, while energy requirements was equal to 1.29 UFL (0.69 UFL, maintenance plus 0.60 UFL, milk production). The lack of 0.53 UFL was guaranteed by the concentrates. As concerns groups H and L, the energy requirements have been satisfied by the intake of 1.2 kg as fed of alfalfa hay plus concentrates.

Table 2 shows the chemical composition and the fatty acid profile of pasture along the trial.

Table 2. Chemical composition and fatty acid profile of pasture along the trial

	April	May	June	July	September
	Chemical composition, % DM				
Crude Protein	16.6	17.2	16.7	14.9	17.9
Ether Extract	2.0	2.0	2.0	2.0	1.9
NDF	50.0	49.2	49.0	51.6	45.5
ADF	32.4	32.6	33.3	34.6	30.5
ADL	4.8	4.8	4.9	5.3	4.4
	Fatty acid profile, % of total FA				
SFA	20.7	18.0	15.7	19.7	14.0
MUFA	9.0	4.6	4.5	4.3	4.4
PUFA	71.4	78.3	79.0	76.7	82.3
Linoleic acid	10.4	22.9	35.3	21.9	48.0
Linolenic acid	31.2	41.0	43.8	40.4	52.0

SFA = saturated fatty acids; MUFA = mono-unsaturated fatty acids; PUFA = poly-unsaturated fatty acids.

The worst chemical composition of pasture was registered in July while in May and September, the pasture showed higher protein and lower NDF value. The sample collected in September had the lowest values of saturated fatty acid (SFA) and the highest values of linoleic and linolenic acids.

The body weight of all the groups did not change along the trial (Table 3).

Table 3. Live weight (LW; kg), milk yield (MY, g/head/d) and composition (%)

Group	LW	MY	Protein	Fat	Lactose
	Group effect (G)				
H	50.1	1428	3.60	3.70^{B}	4.65^{a}
P	49.8	1337	3.57	4.62^{A}	4.57^{b}
L	49.9	1432	3.58	3.90^{B}	4.61^{a}
	Month of lactation effect (S)				
1	49.2	2040^{A}	3.30^{C}	4.22^{B}	4.75^{a}
2	49.6	1700^{B}	3.52^{BC}	4.53^{A}	4.75^{a}
3	50.3	1365^{C}	3.58^{B}	4.00^{C}	4.56^{b}
4	49.8	1158^{C}	3.40^{BC}	4.20^{B}	4.53^{b}
5	50.8	718^{D}	4.10^{A}	4.55^{A}	4.48^{b}
G*S	NS	NS	NS	NS	*
SEM	1.30	37.49	0.15	0.37	0.11

A, B, C, D: P< 0.01; a, b and *: P < 0.05; NS: not significant, SEM: standard error of mean.

GS: interaction between main effects.

Average milk yield did not statistically differ among the groups while it significantly decreased as days in milk increased. Groups P showed significantly higher fat content than group H and L (4.62% *vs* 3.70%, and 3.90% respectively for groups P, H and L; P < 0.01). On the contrary, milk from goats of group H and L had significantly higher content of lactose (4.65% and 4.61% *vs* 4.57%, respectively for groups H, L and P; P < 0.05). As expected, significant differences were found as function of sampling month also for the milk qualitative parameters.

As depicted in Table 4, the levels of C18:1 *cis9*, C18:1 *trans11* and MUFA in milk were significantly (P < 0.05) increased by both the pasture and linseed, while linoleic acid (C18:2) and PUFA were significantly (P < 0.05) higher in group P compared either to H or to L groups.

Table 4. Milk fatty acid profile (g/100 g of fat)

Fatty acid	H	P	L	SEM
C4:0	2.50	2.37	2.41	0.10
C6:0	3.01	3.10	3.02	0.10
C8:0	2.89	3.01	3.00	0.14
C10:0	12.2	11.2	11.3	0.32
C12:0	3.88	3.34	3.40	0.12
C14:0	8.44	8.00	8.21	0.24
C14:1	0.53	0.57	0.54	0.01
C16:0	19.4	18.8	19.0	0.33
C16:1	1.44	1.30	1.39	0.04
C17:0	0.62	0.67	0.66	0.01
C17:1	0.26	0.26	0.26	0.01
18:0	8.10	7.48	8.00	0.21
C18:1cis9	15.4^{b}	16.6^{a}	16.4^{a}	0.21
C18:1trans11	1.4^{b}	1.8^{a}	1.7^{a}	0.04
C18:2	2.01^{b}	2.31^{a}	2.10^{b}	0.10
C20:0	0.22	0.22	0.22	0.01
C18:3	0.64	0.74	0.66	0.02
C20:2	0.20	0.21	0.20	0.01
C22:0	0.17	0.17	0.16	0.01
C20:3	0.49	0.49	0.49	0.02
C20:4	0.26	0.25	0.27	0.01
c9t11CLA	0.510^{Bc}	0.780^{Aa}	0.648^{b}	0.04
t10c12CLA	0.031^{Bc}	0.051^{Aa}	0.044^{b}	0.8×10^{-3}
c9c11CLA	0.017	0.021	0.018	0.8×10^{-4}
ΣCLA	0.55^{Bc}	0.87^{Aa}	0.70^{b}	0.03
SFA	62.3	60.6	61.0	0.60
MUFA	19.1^{b}	22.3^{a}	21.2^{a}	0.41
PUFA	3.67^{b}	4.00^{a}	3.72^{b}	0.44

A, B: $P < 0.01$; a, b: $P < 0.05$; SEM: standard error of mean;

SFA = saturated fatty acids; MUFA = mono-unsaturated fatty acids;

PUFA = poly-unsaturated fatty acids.

The highest values of CLAs, either as *t11* CLA and *t10 c12* CLA or as ΣCLA, have been registered in milk of group P, the lowest in group H. These parameters in milk of group L were significantly ($P < 0.05$) lower or higher than those of group P and H, respectively. In contrast with the results of Valvo, Bella, Scerra and Biondi (2007) which found lower values of saturated fatty acids (SFA) in milk of grazing sheep than in milk of sheep housed in stall, in present trials this parameter was not different among the groups,

CLA contents and fatty acid profile in milk of group P were significantly ($P < 0.01$) affected by the sampling month (Table 5). In particular, the highest values of PUFA, *c9 t11* CLA, *t10 c12* CLA, *c9 c11* CLA and ΣCLA were registered in June and September.

Table 5. Milk fatty acid profile and CLAs contents (mg/100 g of fat) in grazing group along the trial

	April	May	June	July	September	SEM
SFA	58.0^{B}	60.4^{A}	59.3^{AB}	58.2^{B}	60.1^{A}	0.48
MUFA	22.0	22.5	21.6	21.3	21.8	0.41
PUFA	3.3^{B}	3.6^{B}	4.9^{A}	3.3^{B}	4.9^{A}	0.22
c9t11CLA	0.454^{D}	0.782^{C}	0.911^{B}	0.741^{C}	1.077^{A}	0.02
t10c12CLA	0.025^{D}	0.058^{A}	0.062^{A}	0.041^{C}	0.050^{B}	1.2×10^{-3}
c9c11CLA	0.001^{C}	0.019^{B}	0.035^{A}	0.023^{B}	0.033^{A}	0.5×10^{-4}
Σ CLA	0.478^{C}	0.849^{B}	0.106^{A}	0.818^{B}	1.122^{A}	0.02

SFA = saturated fatty acids; MUFA = mono-unsaturated fatty acids; PUFA = poly-unsaturated fatty acids; A, B: $P < 0.01$; SEM: standard error of mean.

4. Discussion

The positive effect of pasture on milk fat has been reported also by other authors. In a study carried out on Alpina goats, Soryal, Zeng, Min and Hart (2004) registered higher fat percentage in milk of grazing group than in that of group housed in stable and fed alfalfa hay; similar results are reported by D'Urso, Cutrignelli, Calabrò, Bovera, Tudisco, Piccolo and Infascelli (2008). Concerning fatty acid profile, SFA levels have not been affected by feeding system, as reported also by Nudda et al. (2006) for linseed supplementation of diet. According to Chilliard and Ferlay (2004) the result could be attributed to the fact that they are partly synthesized by metabolic pathways that are independent of acetyl-CoA carboxylase. In contrast to dairy cows, enzymes involved in the pathways of de novo lipogenesis in the goat mammary gland seemed less affected by the lipid supplementation with PUFA.

Milk of group P and L showed significantly increase ($P < 0.05$) of C18:1 isomers. It is well known that linoleic and linolenic acids are biohydrogenated in the rumen. Even if the predominant end product would be stearic acid, several monounsaturated intermediates are often generated (Sanz-Sampelayo et al., 2007) and thereafter found in the milk. In present trial, among PUFAs, the linoleic acid was significantly higher in milk of grazing group compared to control (H) group. This result could be attributed to the higher content of linoleic acid in the pasture compared either to the alfalfa hay (23.9 vs 17.1% of total fatty acids). Cabiddu, Addis, Spada, Sitzia, Molle and Piredda (2004) found that the overall PUFA content in milk from sheep grazing pastures rich in legumes was higher (on average 6% of total fatty acids) than that of stall-fed sheep (4.07% of total fatty acids). The highest values of PUFA in milk of group P were registered in June and September, according to the fatty acid profile of pasture along the trial as reported also by Tsiplakou, Mountzouris and Zervas (2006) on grazing sheep. Both linoleic and linolenic acids levels in milk fat did not significantly increase when linseed were supplemented, according to Mir, Goonewardene, Okine, Jaegear and Scheer (1999), Chilliard et al. (2006), Nudda et al. (2006).

In present trial, pasture and linseed supplementation of diet highly affected the CLA concentration of goat milk as reported respectively by Sanz Sampelayo et al. (2007) and Luna, Bach, Juarez and de la Fuente (2008). The higher value of *c9 t11* CLA and *t10 c12* CLA found in the milk of groups P and L could be attributed to the higher level found in the pasture and in concentrate fed by group L of linoleic and linolenic acids, which are recognised as their main precursor (Kemp & Lander, 1984; Kim et al., 2000). In addition, the lower content of *c9 t11* CLA in milk of goats housed in stall, may also related to the loss of precursor fatty acids during the hay making process (Aii et al., 1988).

Also the different levels of CLAs by sampling month in milk of group P, agree with that of their precursors in the pasture. However, particularly the *c9 t11* CLA isomer originate also by endogenous synthesis by the Δ^9 desaturase in the mammary gland, starting from the transvaccenic acid which is an intermediate product of linoleic acid bioidrogenation in the rumen (Grinarii & Bauman, 1999). According to Lock and Garnsworthy (2003) part of increase of *c9 t11* CLA in milk may be due to the apparent increase in activity of Δ^9 desaturase in grazing animals.

5. Conclusion

The favourable influence of pasture on the nutritional value of goats milk has been confirmed, as the group at pasture showed significantly higher levels of MUFA, linoleic acids and CLAs, widely recognised as having beneficial effects on human health. Similarly, feeding linseed to lactating goats changed fatty acid profile increasing MUFA and CLA levels, even if the values of CLAs were significanly lower compared to those found in milk op grazing group. Finally, it has to be underlined that the improvements in milk fatty acid profile has been achieved without detrimental effects on milk yield.

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