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Effect of tomato by-products in the diet of Comisana sheep on composition and conjugated linoleic acid content of milk fat

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ABSTRACT

To evaluate the effect of supplementing the diet of Comisana sheep with by-products from industrial tomato manufacture on the fat composition and conjugated linoleic acid (CLA) content of milk fat, two groups of 50 ewes each were fed either total mixed ration standard (TMRS) or total mixed ration with added tomato by-products (TMRA). Milk fat composition was determined by high-resolution gas chromatography (HRGC). The milk fat content for the animals fed the TMRA diet increased by 6.41% ($P < 0.01$) after six weeks, compared with the animals fed the TMRS diet. The CLA content in the milk fat for the group of animals fed the TMRA diet was 19.8% ($P < 0.05$) higher than for those fed the TMRS diet, and reached 1.33 g 100 g^{-1} fat, while the polyunsaturated fatty acid (PUFA) content increased by a 6.43% ($P < 0.05$) and reached 7.12 g 100 g^{-1} fat. The fatty acid composition showed an increase in the amount of polyunsaturated fatty acids. The n – 3:n – 6 ratio increased by 13% in the milk from sheep fed with the TMRA diet. These observations were confirmed by triglyceride analysis, which showed a decrease in the amount of short-chain (C_{28}–C_{32}) and medium-chain (C_{34}–C_{42}) triglycerides after six weeks, while the opposite was observed for the long-chain triglycerides (C_{44}–C_{54}).

1. Introduction

Comisana sheep are one of the most important breeds of Mediterranean sheep, and this breed is raised for its high milk yield, and usually processed on site to produce cheeses, e.g., Tuma, Primo Sale and Pecorino Siciliano. The milk has an average fat content of 8.5% (w w^{-1}), a protein content of 5.2% (w w^{-1}) and gives a high cheese yield (Zhang, Mustafa, & Zhao, 2006). Feeding the animals with fat-containing sources can modify the fatty acid composition of the produced milk (Kelly, Kolver, Bauman, Van Amburgh, & Muller, 1998; Kennelly, 1996). The introduction into the rumen of unsaturated fatty acids that can be hydrogenated by anaerobic microorganisms produces conjugated linoleic acids (CLAs); these compounds are a mixture of positional and geometric isomers of linoleic acid, *cis*-9, *cis*-12 octadecadienoic acid, that contain conjugated double bonds, indicated as C18:2c9,c12 in the following. Rumenic acid, *cis*-9, *trans*-11 octadecadienoic acid, RA, indicated also as C18:2c9, t11 is considered to be the primary form of CLA naturally present in food products, and this isomer accounts for 80% of total CLA isomers (Park, 2009).

The richest dietary source of CLA is milk fat. CLAs are capable of exhibiting health benefits, including anticarcinogenic, antibacterial, antiatherosclerotic, free-radical scavenging, and immunomodulatory effects, as well as altering tissue fatty acid composition and metabolism, influencing signal transduction, and antibacterial activities (Jahreis et al., 1999; Macdonald, 2000; Parodi, 1999; Sieber, Collomb, Aeschlimann, Jelen, & Eyer, 2004; Yu, Adams, & Watkins, 2003). A CLA-rich diet has been observed to have positive health effects, e.g., enhancing growth, and reducting body fat (Dhiman et al., 2000; Pariza, Park, & Cook, 2001); however, it is worth noting that most of the health effects were observed only in animal experiments up to now, and results of human studies are contradicting (Sailas & Spener, 2009).

The mechanism of formation of CLA can be attributed to the action of anaerobic microorganisms such as *Butyrivibrio fibrisolvens* (Kepler, Hirons, Mc Neill, & Tove, 1966) and CLA can be also produced through the action of *Lactobacillus reuteri*, *Proprionibacterium*, *Bifidobacterium* and *Enterococcus*, which are able to transform linoleic acid into CLA (Pariza et al., 2001). CLA in ruminant milk can derive both directly and indirectly from incomplete microbial hydrogenation of polyunsaturated fatty acids (PUFAs) in the rumen (Bauman, Baumgard, Corl, & Griinari, 1999). Anaerobic bacteria can produce CLA as an intermediate in the

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biohydrogenation of linoleic acid (Griinari & Bauman, 1999) and CLA is also formed from desaturation of vaccenic acid, *trans*-11 octadecenoic acid, VA, also indicated as C18:1t11, in the mammary gland via delta-9 desaturase enzyme (Griinari et al., 2000). The variations in CLA content in milk have been associated with several factors, such as stage of lactation, parity (Kelly et al., 1998), and breed (Secchiari et al., 2001; White et al., 2001). However, diet remains the most important factor influencing milk CLA concentration (Collomb, Butikofer, Sieber, Jeangros, & Bosset, 2002); in fact, the highest CLA levels in milk fat have been obtained from fresh pastures or diets supplemented with vegetable oils naturally rich in PUFA (Loor, Ferlay, Ollier, Doreau, & Chilliard, 2005; Stanton, Murphy, McGrath, & Devery, 2003).

Sunflower, linseed, canola oil, and graded quantities of dietary-rumen-protected fat have been added to animal diets to enrich the fatty acid composition of the milk (Rotunno, Sevi, Di Caterina, & Muscio, 1998) and are widely used in animal nutrition to increase the energy density of the diet. They may be added in the form of calcium soaps, palm oil fatty acids, or natural protected fats like seeds. Tomato processing is one of the main food industries in Southern Italy and the by-products of these activities, seeds and skin, are usually discarded or pulverized to be included in animal feed (Ashes et al., 1992). The seeds contain about 27% extractable lipids and these have been identified as edible oils containing oleic and linoleic acids as the predominant fatty acids. Tomato skin is a protein-rich source that contains about 11% protein (Al-Wandawi, Abdul-Rahman, & Al-Shaikhly, 1985; Del Valle, Camara, & Torija, 2003).

This paper describes the effect of a diet supplemented with tomato by-products (seeds and peel) on the composition of milk of Comisana sheep, measured by evaluating contents of CLA, fatty acids, triglyceride and cholesterol, using high-resolution gas chromatography (HRGC).

2. Materials and methods

2.1. Collection and preparation of samples

One hundred Comisana sheep raised in the area of Benevento, Southern Italy, were divided into two groups (50 animals each) fed with two diets of different composition. Each group consisted of animals at the same lactation stage with a body condition score (BCS) between 2.75 and 3.25 (Russel, 1984), free from mastitis or any other inflammatory disease. The trial lasted 6 weeks, and milk sampling was performed during the summer and autumn of the year 2008. One group of animals were fed with a total mixed ration standard diet, TMRS, whereas the other received a total mixed ration diet with added tomato processing by-products, TMRA. Milk samples from each group, indicated as “additive milk”, AM, from sheep fed the TMRA diet and “standard milk”, SM, from sheep fed the TMRS diet, were collected every 7 days for a total time of six weeks. The samples were collected from each animal using 250-mL sterile glass containers, refrigerated and transferred to the laboratory for determination of fat content and chromatographic analysis. All samples were collected and the analyses were conducted in duplicate. The total content of lipids, lactose and proteins in milk samples were determined using a MilkoScan™ Minor 4 apparatus.

2.2. Materials

For the laboratory analyses, a MilkoScan™ Minor 4 (Foss Electric, Hillerod, Denmark), a PK 131 centrifuge (ALC International, Milan, Italy), a Laborota 4000 rotating evaporator (Heidolph, Milan, Italy), and a 2055 Soxhlet extractor (Foss Electric, Hillerod, Denmark), were used. For the chromatographic analysis, a gas chromatograph Autosystem XL (Perkin Elmer, Norwalk, CT, USA) equipped with a Programmed Split-Splitless (PSS) injector and Flame Ionization Detector (FID), connected to Turbochrom version 4.1 data acquisition system, and a Gas chromatograph DANI 8521-a (Dani, Monza, Italy) equipped with injector Programmed Temperature Vaporizer (PTV) and FID detector, connected to a Clarity acquisition system (Palo Alto, CA, USA), were used. All solvents and reagents were of an analytical grade and were purchased from Fluka (Buchs, Switzerland). Chromatographic grade helium and hydrogen gases were purchased from SON (Naples, Italy).

2.3. Diet, composition and fatty acids analysis

The chemical composition of the two experimental diets, standard ration (TMRS) and ration added with tomato by-products (TMRA), was determined according to the methods described by AOAC (2005) and Goering (1970). The extracted fat was converted to methyl esters and analyzed by gas chromatography following the procedure reported in Section 2.4. Peak identification and quantification was performed as described for fatty acid (FA) analysis.

2.4. Gas chromatographic analysis of fatty acids and conjugated linoleic acid

Lipids were extracted from milk samples by the Röse-Gottlieb method (FIL-IDF, 1996). Milk FA composition was determined after the *trans* esterification of triglycerides into the fatty acid methyl esters (FAME) by reaction with potassium hydroxide in methanol (Ichihara, Shibahara, Yamamoto, & Nakayama, 1996) with minor modifications. One hundred milligrams of extracted fat was weighed in a glass-stoppered 10 mL test-tube and dissolved in 2 mL of n-hexane; 300 μL of 2 M KOH in MeOH solution were added. After stirring for 2 min at room temperature, and allowing the two phases to separate, 1 μL of the upper phase was injected into the gas chromatograph. A Perkin Elmer Autosystem XL gas chromatograph equipped with a fused silica capillary column SP 2380 (Supelco, Bellefonte, USA) 100 m × 0.25 mm i.d.; 0.20 μm film thickness, was used. The column was held at 100 °C for 5 min after injection, heated at 3 °C min^{-1} to 165 °C, held at 165 °C for 10 min, and then heated at 3 °C min^{-1} to 260 °C and held at the final temperature for 28 min.

The injector PSS temperature was initially set at 50 °C for 0.1 min, increased at 400 °C min^{-1} up to 260 °C and held for 10 min. Split ratio was 1:60, and gas carrier (H_2) flow was set at 20 cm s^{-1}, FID temperature was set at 260 °C.

Supelco 37 Component Fame Mix (Supelco Bellefonte, PA, USA) and CLA isomer mixture (Nu-Chek Prep., Inc. Elysian, MN, USA) were used as external standards for FA and CLA peak identification, respectively. Published gas chromatography (GC) retention data (Ledoux et al., 2005) were also used as reference. A certified reference material (CRM) 164 milk fat reference supplied by the Community Bureau of Reference (Commission of the European Communities, Brussels, Belgium) was used to obtain correction factors (CF) and to convert area percentage for individual FA into weight percentages.

2.5. Gas chromatographic analysis of triglycerides and cholesterol

About 0.2 g of extracted milk fat was weighed to the nearest 0.1 mg and diluted in 10 mL of n-hexane; 1 μL of this solution was injected into the gas chromatograph. Analyses were performed on a GC Dani 1000 gas chromatograph equipped with a capillary column (30 m × 0.25 mm i.d.; 0.10 μm) Rtx 65-TG (Restek, Bellefonte, PA, USA). The column was held at 250 °C for 2 min after injection, heated at 6 °C min^{-1} up to 360 °C and held for 15 min.

The PTV was held in split mode with a splitting ratio of 1:60 and the following temperature program: 50 °C for 0.1 min, then 400 °C min^{-1} up to 370 °C, and holding for 5 min. The flow-rate of the gas carrier, He, was 1.2 mL min^{-1} and FID temperature was set at 360 °C.

Triglycerides were identified by their retention times using a certified reference anhydrous milk fat, CRM 519, supplied by the Community Bureau of Reference (Commission of the European Communities, Brussels, Belgium). Correction factors (CF) for converting the area percentages into weight percentages were calculated. The reference standard for cholesterol was purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.6. Statistical analysis

Data were analyzed using the General Linear Model (GLM) procedure of Statistical Analysis System package (SAS Inst. Inc., Cary, NC, USA). The data set included 14 objects (milk samples) and 31 variables (milk fatty acid composition, milk triglyceride composition and milk fat percentage). Data were analyzed by one-way analysis of variance with treatment (TMRS and TMRA) as factor; a *t* test was used to identify differences between least square means. Significance was determined at $P < 0.05$.

3. Results and discussion

Table 1 shows the ingredients and chemical composition of the two diets used to feed the two groups of Comisana ewes. TMRA contained about 20 g 100 g^{-1} dry matter of tomato processing by-products and, compared with the TMRS, had reduced amounts of barley straw, soybean meal and wheat bran. TMRA had similar chemical composition to the TMRS, having notably the same energy content, but a marked difference in the amount of lipids. Moreover, the FA composition of the TMRA showed a higher percentage of unsaturated fatty acids, at about 75 g 100 g^{-1}, compared to the TMRS diet, which contained about 56 g 100 g^{-1} (see Table 1). The milk yield, lactose, and total solids content of the milk were not affected by the diet.

The average protein contents were similar in the two types of milk (46.8 g L^{-1} in SM and 47.1 g L^{-1} for the AM respectively), which was as expected, this value being genetically controlled (Rotunno et al., 1998). On the other hand, the milk produced by the sheep receiving the TMRA diet showed a higher lipid content than milk produced by the sheep fed with TMRS diet.

After 6 weeks, the total lipid content of the milk from the 50 animals fed with the TMRA diet was 0.22 kg, showing an increase from 7.8% (w w^{-1}) to 8.3% (w w^{-1}) ($P < 0.01$). The sheep fed with the two diets did not suffer from any nutritional stress: diet formulation had a balanced composition of all nutrients.

The average FA concentration, as well as the distribution of saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids is summarized in Table 2. The effect of the addition of tomato by-product to the diet was significant for some of the FAs. Lower contents of decanoic acid, C10:0, and palmitic acid, C16:0 ($P < 0.05$), and a higher content of stearic acid, C18:0 ($P < 0.05$), were measured in the milk fat from ewes fed with TMRA than in the milk from ewes fed with TMRS diet. The FA content

Table 1
Ingredients and composition of the two experimental diets fed to ewes.[a]

	Total mixed ration standard	Total mixed ration added
Ingredients (g 100 g^{-1} DM)		
Wheat bran	14.90	8.30
Maize	18.00	19.70
Beetroot pulp	11.70	11.40
Soybean meal	16.60	15.30
Barley straw	36.50	22.70
Vitamin supplement	1.80	1.80
Hydrogenated fat	0.50	0.50
Tomato by-products	–	20.40
Chemical composition (g 100 g^{-1} DM)		
Dry matter	88.2	82.0
Crude protein	14.28	16.06
EE	2.79	5.82
NDF	49.30	47.70
Milk forage units kg^{-1}	0.89	0.88
Metabolizable energy (MJ kg^{-1})	11.72	12.18
Fatty acid composition (g 100 g^{-1} fat)[a]		
C14:0	2.02 ± 0.32^{A}	0.12 ± 0.21^{B}
C16:0	31.19 ± 0.65^{A}	16.09 ± 0.55^{B}
C16:1	0.63 ± 0.21	0.63 ± 0.97
C17:0	1.91 ± 0.69^{A}	0.32 ± 0.66^{B}
C18:0	5.54 ± 0.32^{a}	6.54 ± 0.41^{b}
C18:1 *cis*	20.86 ± 0.11	22.86 ± 0.21
C18:2	33.08 ± 0.33^{A}	49.08 ± 0.12^{B}
C18:3	2.59 ± 0.99	2.99 ± 0.59
C20:0	0.54 ± 0.25	0.51 ± 0.66
∑MUFA	21.49 ± 0.32	23.49 ± 1.18
∑PUFA	35.67 ± 1.32	52.07 ± 0.71

[a] Abbreviations are: DM, dry matter; EE, ether extract (fat, sterols, carotenoids); NDF, neutral detergent fiber; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

[b] Values ± standard deviation with different superscripts differed significantly: A, B indicate a significant difference at $P < 0.001$; a, b indicate a significant difference at $P < 0.05$.

Table 2
Fatty acid composition (g 100 g^{-1} fat ± standard deviation)[a] in milk of Comisana sheep fed with total mixed ration standard (TMRS) and a total mixed ration diet with added tomato by-products (TMRA) for 6 weeks.[b]

FA	TMRS	TMRA	Significance[c]
C4:0	3.61 ± 0.14	3.34 ± 0.13	NS
C6:0	2.51 ± 0.13	2.28 ± 0.12	NS
C8:0	2.24 ± 0.11	2.15 ± 0.13	NS
C10:0	7.01 ± 0.17	6.64 ± 0.16	**
C12:0	3.74 ± 0.44	3.83 ± 0.65	NS
C14:0	9.61 ± 0.29	10.04 ± 0.10	NS
C15:0	0.33 ± 0.13	0.30 ± 0.22	NS
C16:0	25.04 ± 0.15	24.50 ± 0.14	**
C17:0	0.55 ± 0.09	0.54 ± 0.07	NS
C18:0	10.23 ± 0.15	11.04 ± 0.11	**
C20:0	0.31 ± 0.04	0.33 ± 0.03	NS
C22:0	0.45 ± 0.01	0.37 ± 0.02	NS
C23:0	0.55 ± 0.01	0.48 ± 0.01	NS
∑SFA	66.18 ± 1.23	65.84 ± 1.20	NS
C14:1	0.82 ± 0.01	0.78 ± 0.03	NS
C16:1	0.98 ± 0.02	0.94 ± 0.01	NS
C17:1	0.19 ± 0.03	0.21 ± 0.02	NS
C18:1 *cis*	21.44 ± 0.21	22.11 ± 0.22	NS
C18:1 *trans*-11 (VA)	2.56 ± 0.04	2.01 ± 0.03	**
∑MUFA	25.99 ± 1.07	26.05 ± 1.04	NS
C18:2 n6	2.78 ± 0.03	2.88 ± 0.03	**
C18:3 n3	1.16 ± 0.04	1.50 ± 0.02	**
C20:2 n9	0.52 ± 0.05	0.54 ± 0.06	NS
C20:3 n6	0.71 ± 0.07	0.73 ± 0.05	NS
C20:4 n6	0.54 ± 0.01	0.51 ± 0.03	NS
C20:5 n3	0.17 ± 0.02	0.18 ± 0.02	NS
C22:6 n3	0.81 ± 0.04	0.78 ± 0.01	NS
∑PUFA	6.69 ± 0.13	7.12 ± 0.10	**
∑n − 3/∑n − 6	0.53 ± 0.02	0.60 ± 0.03	**
∑CLA	1.11 ± 0.01	1.33 ± 0.02	**
∑UFA/∑SFA	0.51 ± 0.04	0.52 ± 0.05	NS

[a] n = 100 (50 samples in duplicate) for each group of Comisana sheep ± standard deviation.

[b] Abbreviations are: FA, fatty acid; SFA ¼ saturated FA; VA, vaccenic acid; MUFA, monounsaturated FA; PUFA, polyunsaturated FA; CLA, conjugated linoleic acid, P (C18:2 c9, t11; C18:2 c10, t12); UFA, unsaturated FA.

[c] NS, non-significant at $P > 0.05$, **significant at $P < 0.05$.

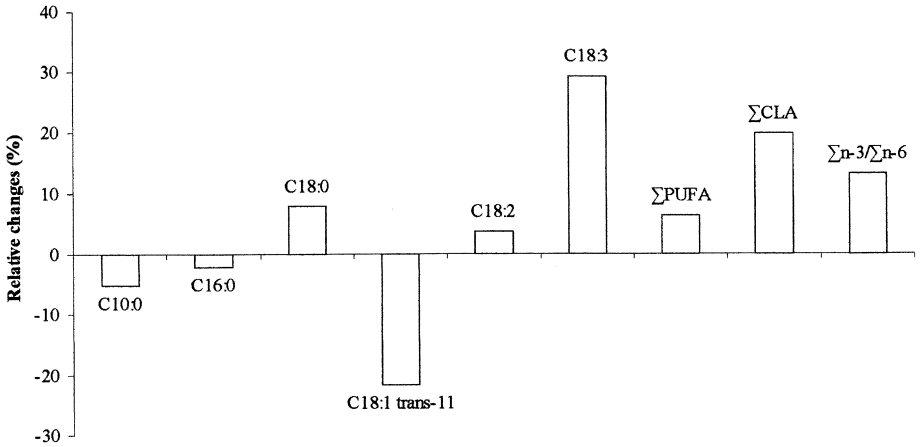

Fig. 1. Changes (%) after six weeks in the composition of the significantly different fatty acids (FAs) in milk from Comisana sheep fed with the total mixed ration supplemented with tomato processing by-products (TMRA) diet relative to the FA composition in milk from sheep fed with the total mixed ration standard diet (TMRS). PUFA, polyunsaturated fatty acid; CLA, conjugated linoleic acid; VA, C18:1 *trans*-11.

(Table 2) indicated that the milk produced by the group of sheep fed with the TMRA diet had a higher content of PUFA ($P < 0.05$) after 6 weeks than that of the sheep fed the TMRS diet. In particular, the concentration of linoleic acid in AM was significantly higher ($P < 0.05$), as were the concentrations of both the isomers of linolenic acid, C18:3 ($P < 0.05$).

The n − 3/n − 6 FA ratio changed from 0.53 to 0.60 ($P < 0.05$). Increased amounts of linoleic and linolenic acids in the milk are considered to be important for their beneficial effects in the prevention of cardiovascular diseases and hypertension in humans (Parodi, 2004). The presence of a higher content of polyunsaturated fatty acids in the milk from ewes fed tomato by-products as a source of linoleic acid is consistent with previously reported data (Zhang et al., 2006). The PUFA content of the milk can be increased by using PUFA-rich feeds such as oilseeds, and their level in milk depends on the amount of these FAs that pass through the rumen. A fraction of linoleic and linolenic acids can escape in the biohydrogenation rumen and could be incorporated into the triglycerides of milk fat in the mammary gland (Luna, Bach, Juareza, & De la Fuente, 2008).

Feeding tomato by-products to the sheep in the TMRA diet could have an added partial protective effect on the lipids against ruminal biohydrogenation (Ashes et al., 1992). The non-protected fraction, containing linoleic acid (C18:2c9,c12) and both α-linolenic (C18:3c9,c12,c15) and γ-linolenic (C18:3c6,c9,c12) acids, was exposed to the action of multiple rumen microorganisms, notably *B. fibrisolvens* (Kepler et al., 1966). These microorganisms primarily transform linoleic acid, α-linolenic, and γ-linolenic acids to RA, and *trans* vaccenic acid VA. The linoleate *cis*-12, *trans*-11-isomerase of *B. fibrisolvens* transposes the double bond at position 12 of linoleic acid to position 11, and modifies it into the *trans* configuration to produce RA. Some of this RA can escape further ruminal biohydrogenation, enter the blood circulation for transport and storage in peripheral tissues and be available for the milk fat synthesis. RA can be further hydrogenated to VA and then to stearic acid; some of this VA can likewise exit the rumen and pass into the blood circulation. On the other hand, α-linolenic and γ-linolenic acid cannot be directly converted to RA: they are hydrogenated to VA, which adds to the VA pool, largely present in peripheral tissue (including the mammary gland), where it is desaturated by delta-9

Table 3
Triglyceride and cholesterol contents (g 100 g^{-1}),[a] after 1 and 6 weeks, in milk of Comisana sheep fed with total mixed ration standard (TMRS) or total mixed ration with added tomato by-products (TMRA).

Triglycerides	One week		Six weeks			
	TMRS	TMRA	TMRS	TMRA	*P*-Values	Relative changes (%)[b]
C28	1.35 ± 0.08	1.36 ± 0.12	1.32 ± 0.13	1.13 ± 0.12	<0.001	−14.50
C30	2.71 ± 0.19	2.68 ± 0.33	2.67 ± 0.23	2.21 ± 0.25	<0.001	−17.10
C32	4.86 ± 0.32	4.78 ± 0.32	4.42 ± 0.42	4.00 ± 0.32	<0.05	−9.50
C34	8.23 ± 0.23	8.15 ± 0.86	7.82 ± 0.26	7.30 ± 0.95	<0.05	−6.20
C36	12.56 ± 0.29	12.38 ± 0.85	12.64 ± 0.32	11.66 ± 0.83	<0.05	−8.00
C38	15.11 ± 0.66	15.16 ± 0.70	14.28 ± 0.62	14.66 ± 0.80	<0.001	+2.28
C40	14.50 ± 0.21	14.80 ± 0.43	14.67 ± 0.23	13.89 ± 0.42	<0.001	−5.40
C42	9.92 ± 0.17	10.04 ± 0.33	9.80 ± 0.22	9.85 ± 0.28	<0.001	+0.65
C44	7.81 ± 0.59	8.01 ± 0.28	7.74 ± 0.51	7.64 ± 0.31	<0.05	−3.22
C46	5.92 ± 0.28	6.08 ± 0.41	6.12 ± 0.32	7.09 ± 0.36	<0.001	+11.57
C48	4.82 ± 0.23	5.08 ± 0.70	5.02 ± 0.21	5.98 ± 0.62	<0.001	+19.16
C50	4.74 ± 0.58	4.87 ± 0.80	5.46 ± 0.52	6.11 ± 0.80	<0.05	+7.67
C52	4.94 ± 0.83	4.85 ± 1.42	5.54 ± 0.85	5.76 ± 1.23	<0.05	+4.00
C54	1.64 ± 0.28	1.73 ± 0.14	1.60 ± 0.28	1.99 ± 0.19	<0.001	+24.50
Cholesterol	TMRS	TMRA	TMRS	TMRA	*P*-Values	Relative changes (%)[b]
	0.39 ± 0.02	0.35 ± 0.04	0.39 ± 0.09	0.33 ± 0.07	<0.001	−16.10

[a] n = 100 (50 samples in duplicate) for each group of Comisana sheep ± standard deviation.
[b] Changes in the triglyceride composition and cholesterol content in milk fat of the TMRA group relative to the composition of lipids in milk of the TMRS group after six weeks.

desaturase to form RA (Bauman et al., 1999; Bauman & Griinari, 2003; Griinari & Bauman, 1999; Griinari et al., 2000; Luna et al., 2008).

A higher content of CLA was found in the milk from ewes fed with TMRA diet compared with the milk from ewes fed with the TMRS diet (Table 2; $P < 0.05$). Data reported refer to total CLA, considering the *cis*-9, *trans*-11 CLA and *cis*-10, *trans*-12 CLA isomers. This result is in agreement with data reported in the literature (Luna et al., 2008; Zhang et al., 2006). Significantly positive differences in the concentration can also be observed for FAs, e.g., C10:0, C16:0, C18:0, and PUFA. In contrast, VA concentration, as shown in Table 2, was lower ($P < 0.05$) in milk fat from ewes fed TMRA, compared with the TMRS diet. This difference could probably be caused by partial protection against ruminal enzymes responsible for biohydrogenation (Kennelly, 1996; Kepler et al., 1966).

Fig. 1 shows the relative changes for significantly different FAs, PUFA, VA and CLA observed in AM after six weeks. In particular, the content of the C18:3 FA in milk fat from ewes fed the TMRA diet increased by 29.3% compared with TMRS diet; the total CLA content and the n − 3/n − 6 ratio in milk fat from ewes fed with TMRA diet increased by 19.8% and 23.8%, respectively, compared with the TMRS diet. In contrast, a 21.4% decrease in VA concentration was observed (Table 2).

Table 3 shows the average values of the triglycerides and cholesterol content in milk fat after one week and six weeks. The content of short-chain (C_{28}–C_{32}) and medium-chain (C_{34}–C_{42}) triglycerides decreased after 6 weeks, but the opposite can be observed for the long-chain triglycerides (C_{44}–C_{54}), confirming the results of the FA composition reported. Finally, it can be observed that the milk produced by the animals fed with the TMRA diet contains a lower cholesterol level compared with the milk from the animals fed the TMRS diet ($P < 0.001$).

4. Conclusions

Feeding Comisana sheep with by-products from tomato processing significantly altered the composition of milk fat. In particular, the sheep fed with the TMRA diet produced a milk characterized by a higher content of PUFA and n − 3/n − 6 fatty acid ratio. A significantly higher content of CLA and a lower cholesterol content were measured in the milk fat from ewes fed with TMRA diet compared with the TMRS diet. The sheep fed tomato by-products did not suffer from any nutritional stress, considering that the proposed diet formulation had a balanced composition of all nutrients, e.g. crude protein, lipids and fiber. It can be concluded that the addition of tomato processing by-products to the diet of Comisana sheep increases the amount of total fat produced by the animals and positively modifies the milk fat composition. The use of by-products of tomato processing as a dietary supplement for sheep can also contribute to minimizing the negative impact of the tomato industry on the environment, where by-products are usually discarded.

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