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To cite this article: Aldo Prandini, Samantha Sigolo, Carla Cerioli & Gianfranco Piva (2009) Survey on conjugated linoleic acid (CLA) content and fatty acid composition of Grana Padano cheese produced in different seasons and areas, Italian Journal of Animal Science, 8:4, 531-540

To link to this article: <http://dx.doi.org/10.4081/ijas.2009.531>



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Published online: 01 Mar 2016.



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Survey on conjugated linoleic acid (CLA) content and fatty acid composition of Grana Padano cheese produced in different seasons and areas

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Received February 11, 2009; accepted May 7, 2009

ABSTRACT

A study was carried out to determine the content of conjugated linoleic acid (CLA) and fatty acid composition of Grana Padano P.D.O. (Protected Designation of Origin) Italian cheese. Samples were obtained from spring and summer cow's milk in different areas of Grana Padano production district and from all present dairies. In particular, 253 samples of lowland-hill Grana Padano (LH-GP) were analysed: 137 produced from spring milk, and 116 from summer milk. Forty-three mountain Grana Padano (M-GP) samples were also analysed: 25 obtained from spring milk, and 18 from summer milk. The results were subjected to statistical analysis; a factorial arrangement was used, and the fixed effects in the model included season, the manufacturing area of the Grana Padano cheese, and the first order interaction (season x area). Further, cheese β -carotene concentration was measured in a representative number of summer samples (32), selected according to the CLA level (9 with CLA concentration <5; 10 with CLA value ranging from 5 to 7; 13 with CLA level >7 mg/g total fat), in order to investigate the correlation between CLA content and β -carotene concentration.

M-GP had higher levels of CLA, vaccenic (TVA) and α -linolenic (LNA) acid than LH-GP. CLA concentrations increased from spring to summer both in M-GP and LH-GP. This rise was greater in M-GP showing an effect of interaction between season and production area ($P < 0.001$). An effect of first order interaction ($P < 0.001$) was also seen for TVA with values increasing from spring to summer in M-GP, whereas there only was an effect of the manufacturing area on LNA. During summer, M-GP had the highest polyunsaturated fatty acids (PUFA) percentage. An increase in PUFA from spring to summer was observed only in M-GP (first order interaction, $P < 0.001$). The saturated fatty acids (SFA) decreased from spring to summer both in LH-GP and M-GP and with major entity in M-GP (first order interaction, $P < 0.001$). The β -carotene concentration resulted positively correlated with CLA level ($r = 0.92$; $P < 0.01$) in summer cheese. The present results underline the role of mountain cheese obtained from summer milk as a natural source of CLA and beneficial fatty acids for human health.

Key words: CLA, Fatty acid composition, β -carotene, Grana Padano PDO, Cheese.

RIASSUNTO

INDAGINE SUL CONTENUTO DI ACIDO LINOLEICO CONIUGATO (CLA) E COMPOSIZIONE IN ACIDI GRASSI DI FORMAGGIO GRANA PADANO PRODOTTO IN DIFFERENTI STAGIONI ED AREE

È stato condotto uno studio sul contenuto di acido linoleico coniugato (CLA) e composizione in acidi grassi di formaggio Grana Padano DOP (Denominazione di Origine Protetta) ottenuto da latte vaccino primaverile ed estivo nelle differenti aree di tutto il comprensorio di produzione del Grana Padano e di tutti i caseifici presenti.

Sono stati analizzati 253 campioni di Grana Padano prodotto in pianura-collina (LH-GP), di cui 137 ottenuti da latte primaverile e 116 da latte estivo, e 43 campioni di Grana Padano di montagna (M-GP), di cui 25 ottenuti da latte primaverile e 18 da latte estivo.

I risultati sono stati sottoposti ad analisi statistica; è stato usato un disegno fattoriale e gli effetti fissati nel modello includevano la stagione di produzione del latte utilizzato, l'area di produzione del Grana Padano e l'interazione di primo ordine (stagione x area). Inoltre, l'analisi per determinare la concentrazione di β -carotene è stata effettuata su un numero rappresentativo di campioni prodotti da latte estivo (32), selezionati in base al loro contenuto di CLA (9 con una concentrazione di CLA < 5; 10 con un contenuto variante da 5 a 7; 13 con un valore > 7 mg/g di grasso), al fine di valutare il grado di correlazione tra contenuto di CLA e concentrazione di β -carotene.

M-GP ha riportato livelli più alti di CLA, acido vaccenico (TVA) e linolenico (LNA) rispetto LH-GP. Le concentrazioni di CLA aumentavano da primavera ad estate sia in M-GP che in LH-GP. Questo aumento è stato più grande in M-GP mostrando un effetto di interazione tra stagione ed area di produzione ($P < 0,001$). Un effetto di interazione di primo ordine ($P < 0,001$) è stato anche visto su TVA con valori che aumentavano da primavera ad estate in M-GP, mentre c'è stato solo un effetto dell'area di produzione su LNA. In M-GP estivo è stata trovata la più alta percentuale di acidi grassi polinsaturi (PUFA). Un aumento in PUFA da primavera ad estate è stato osservato solo in M-GP (interazione di primo ordine, $P < 0,001$). Gli acidi grassi saturi (SFA) diminuivano da primavera ad estate sia in LH-GP che in M-GP e con maggiore entità in quest'ultimo (interazione di primo ordine, $P < 0,001$). La concentrazione di β -carotene è risultata positivamente correlata con il contenuto di CLA ($r = 0,92$; $P < 0,01$) in Grana Padano estivo.

I risultati ottenuti sottolineano l'importante ruolo del formaggio di montagna prodotto da latte estivo come fonte naturale di CLA e di acidi grassi benefici per la salute umana.

Parole chiave: CLA, Composizione in acidi grassi, β -carotene, Grana Padano DOP, Formaggio.

Introduction

Negative health effects have been attributed to milk and dairy products because of their high levels of saturated fatty acids (SFA), considered risk factors for arteriosclerosis and coronary heart disease, weight gain and obesity (Insel *et al.*, 2004; Lokuruka, 2007). Nevertheless, Haug *et al.* (2007) in a recent review discussed the healthy effects of bovine milk components. Conjugated linoleic acids (CLAs), a group of conjugated octadecadienoic acid isomers that occur naturally in milk, dairy products and beef meat, deserve special attention be-

cause of the anticarcinogenic, antiatherogenic, antidiabetic, immune stimulatory and body fat-reducing properties that they have been reported to possess (Terpstra, 2004; Nagao and Yanagita, 2005; Collomb *et al.*, 2006; Kelley *et al.*, 2007).

In milk from ruminant there is an amount of ruminic acid (*cis*-9, *trans*-11 CLA) due to incomplete biohydrogenation of polyunsaturated fatty acids (PUFA), specially linoleic (LA) and α -linolenic (LNA), in the rumen and from desaturation of vaccenic acid (*trans*-11 C_{18:1}, TVA) in the mammary gland via Δ^9 -desaturase (Bauman *et al.*, 2001; Nagpal *et al.*, 2007).

CLA levels in cow's milk fat range from 2 to 53.7 mg/g of fat (Collomb *et al.*, 2006; Bisig *et al.*, 2007). The variation of CLA content in milk has been associated with several factors. Breed (White *et al.*, 2001; Kelsey *et al.*, 2003), stage of lactation and parity (Kelly *et al.*, 1998) may have small influence on CLA levels. However, cows can exhibit large individual variation in milk CLA concentrations (Peterson *et al.*, 2002; Kelsey *et al.*, 2003). Diet is the most significant factor affecting the CLA content in milk fat. High CLA values occur with diets supplemented with plant or marine oils (Loor *et al.*, 2005; Shingfield *et al.*, 2006) and pasture feeding (Chilliard *et al.*, 2001; Lock and Bauman, 2004). Low CLA levels were associated with richer diets in grass and corn silage or mixed grass/corn silage, and concentrate-based diets (Chilliard *et al.*, 2001; Collomb *et al.*, 2006).

The present study was carried out on the lipidic component of Grana Padano P.D.O. (Protected Designation of Origin) cheese. The aim of the study was to measure the CLA content and fatty acid composition of Grana Padano obtained from spring and summer cow's milk in different areas of the Grana Padano production district (lowland-hill and mountain) considering all the dairies of the Consorzio. The β -carotene concentrations were also measured in a representative number of samples obtained from summer milk in order to investigate the correlation between CLA content and β -carotene concentration.

Material and methods

Sampling

All the Grana Padano cheese samples were supplied by dairies belonging to the *Consorzio per la Tutela del Formaggio Grana Padano* (2009). Hundred-sixty-two dairies, of which 137 were situated in lowland-

hill (altitude 0-500 m) and 25 in mountain (altitude higher than 500 m), supplied us each with 1 sample of Grana Padano obtained from spring milk (March-April 2006). Of those 162 dairies 134, of which 116 were placed in lowland-hill and 18 in mountain, provided us each with 1 sample of Grana Padano obtained from summer milk (July-August 2006). The Grana Padano samples (total 296) were collected at incomplete ripening (6 months aged) and analyzed for fatty acid composition and CLA content. Further, 32 samples of summer cheese were selected according to the CLA level (9 with low CLA concentration, <5mg/g fat; 10 with mean CLA value, range 5-7 mg/g fat; 13 with high CLA level, >7mg/g fat) and analyzed for β -carotene concentration. The correlation between CLA content and β -carotene concentration was then calculated.

All the Grana Padano samples were frozen or vacuum-packed after sampling and stored in a freezer at -18°C or fridge, respectively, until analysis. The analyses were carried out on defrosted samples of finely ground cheese.

Chemical analysis

Lipid extraction from cheese samples was performed under cold conditions using a modified Folch technique (Christie, 1989; Prandini *et al.*, 2007). The lipids were then esterified using the method described by Bannon *et al.* (1985) with modifications (Prandini *et al.*, 2007).

CLA and fatty acid methyl esters were quantified using a GC (Varian 3350) equipped with an automatic sampler (Varian CP-8200), a flame ionization detector (FID) and a CP-Select CB capillary column for FAME (100 m x 0.25 mm i.d.; 0.25 μm film thickness; Chrompack, Varian, Inc., CA). GC oven parameters, gas variables and fatty acid peak identification were as previously described (Prandini *et al.*, 2007).

The CLA content, expressed in mg/g fat, was determined by the following formula:

$$\text{CLA(mg/g fat)} = \left\{ \frac{\text{area}_x \times \text{I.S. (mg)} \times \text{C.F.}_x}{\text{area}_{\text{I.S.}} \times \text{fat(g)} \times 1.04} \right\}$$

where “x” is the CLA isomer, “1.04” the conversion factor from methyl ester to fatty acid and “C.F.” the correction factor obtained from an average of 10 injections of an adequately methylated mixture made up of 0.1 g of conjugated octadecadienoic acid (Sigma Chemical Co, St. Louis, MO) and 0.02 g of tricosanoic acid (I.S.). The C.F. correction factor was determined as follows:

$$\text{C.F.}_x = \left(\frac{\text{area}_{\text{I.S.}}}{\text{area}_x} \right) \times \left(\frac{\text{conc.}_x}{\text{conc.}_{\text{I.S.}}} \right)$$

Gas-chromatographic analysis of FAME considered only the *cis-9,trans-11* CLA isomer among all isomers of CLA. The *cis-9,trans-11* CLA levels were expressed in mg/g fat; the fatty acids were expressed as g/100g of total fatty acids, calculated with peak areas corrected by factors according to AOAC 963.22 method (AOAC, 2000).

β -carotene extraction from cheese samples was performed using the AOAC 43.018-43.023 method (AOAC, 1984) with modifications. β -carotene was quantified by HPLC Thermo Separation Products (TSP) equipped with a TSP-200 pump, an AS-3000 sampling system and a Spectra Focus UV-Vis detector (San Jose, CA, USA). The system was controlled by TSP PCI1000 software. β -carotene elution was monitored at 450 nm and its concentration, calculated on the basis of standard curves prepared with pure standard (Sigma-Aldrich), was expressed as mg/kg of sample.

Statistical analysis

The partial fatty acid composition and CLA content were analysed using a completely randomised design by the GLM proce-

dures of SAS[®] (Statistical Analysis System Institute, 2001). A factorial arrangement was used, and the fixed effects in the model included the production season of the milk, the manufacturing area of the Grana Padano cheese, and the first order interaction (season \times area).

The results were reported as Lsmeans \pm standard error (SE). The significance of the differences among means was evaluated taking $P < 0.05$ as significant.

The correlation between CLA content and β -carotene concentration was estimated by the Pearson correlation coefficient using “proc corr” of the SAS[®].

Results

Partial fatty acid composition and CLA content of Grana Padano cheese

Table 1 shows the partial fatty acid composition and the CLA content of Grana Padano obtained from dairies located at lowland-hill and mountain areas (LH-GP and M-GP, respectively) and produced during spring and summer season.

The CLA concentration was higher ($P < 0.05$) in M-GP (6.52 and 9.47 mg/g fat in spring and summer respectively, $P < 0.05$) compared with LH-GP (5.29 and 5.75 mg/g fat in spring and summer respectively, $P < 0.05$), with values increasing from spring to summer in all analyzed samples but with major entity in M-GP, as shown by the first order interaction ($P < 0.001$).

The TVA and LNA percentages were also greater ($P < 0.05$) in M-GP (TVA=1.19 and 1.66%, $P < 0.05$; LNA=0.58 and 0.61%, in spring and summer, respectively) than in LH-GP (TVA=0.94 and 0.95%; LNA=0.39 and 0.40%, in spring and summer, respectively). An effect of the interaction between production season and area ($P < 0.001$) was seen on the TVA level due to a higher value in summer M-GP than in spring M-GP,

whereas there only was an effect of the GP manufacturing area on the LNA level ($P < 0.001$).

An effect of the GP production area was also seen on the LA content ($P < 0.001$) with greater levels in LH-GP (2.35 and 2.33% in spring and summer, respectively) compared with M-GP (2.07 and 2.21% in spring and summer, respectively). Similar LA percentages were found in the summer samples of LH-GP and M-GP, as displayed by the first order interaction with $P = 0.066$, a value trending to the significance.

Spring and summer LH-GP reported higher oleic acid levels (18.56 and 19.54% respectively, $P < 0.05$) than the corresponding M-GP (17.30 and 18.99% respectively, $P < 0.05$). An increase in oleic acid was seen from spring to summer both in LH-GP and M-GP; this rise was greater in M-GP, as shown by the first order interaction ($P < 0.01$).

Summer M-GP showed the highest PUFA percentage with a statistically significant difference ($P < 0.05$) versus all the other samples (LH-GP, 4.01 and 4.00%; M-GP, 3.97 and 4.38% in spring and summer, respectively). An effect of the interaction between production season and area ($P < 0.001$) was seen on the PUFA level due to a significant increase from spring to summer in M-GP.

The monounsaturated fatty acid (MUFA) contents were lower in the spring samples than in those of the summer both for LH-GP (25.70 and 26.19% in spring and summer respectively, $P < 0.05$) and M-GP (24.17 and 26.08% in spring and summer respectively, $P < 0.05$). A greater increase in MUFA was observed from spring to summer in M-GP, as shown by the first order interaction ($P < 0.001$). Moreover, summer M-GP showed similar MUFA percentage to those of spring and summer LH-GP.

The SFA levels decreased from spring to summer both in LH-GP (65.59 and 64.42% in spring and summer respectively, $P < 0.05$)

and M-GP (67.42 and 64.23% in spring and summer respectively, $P < 0.05$). This SFA drop was more marked in M-GP (first order interaction, $P < 0.001$) but similar SFA contents were found in summer LH-GP and M-GP.

Correlation between β -carotene concentration and CLA content in summer Grana Padano cheese

Figure 1 shows the correlation between β -carotene concentrations and CLA levels measured in 32 Grana Padano samples obtained from summer milk. The β -carotene concentration was expressed in mg/kg of cheese.

During our study we noticed that the samples of summer Grana Padano with the highest CLA levels had a particular yellow colour indicating high β -carotene content. This observation led us to investigate the correlation between β -carotene and CLA contents.

The β -carotene concentration resulted positively correlated with CLA ($r = 0.92$; $P < 0.01$).

It is interesting to underline that the cheese samples reporting CLA content > 7 mg/g fat were all samples collected from dairies in the provinces of Trento and Verona located in mountain areas, except for one sample which was obtained from a certificated organic dairy in a lowland area in the province of Verona. In this sample the highest CLA and β -carotene levels were detected (21.78 mg/g fat and 0.555 mg/kg sample, respectively; data not shown).

Discussion

The results report differences in fatty acid composition and CLA concentration between LH-GP and M-GP, showing an effect of the production area on the CLA, TVA and LNA levels with higher values in M-GP than LH-GP. The causes are probably to investigate

Table 1. Partial fatty acid composition and average CLA content in lowland-hill (LH-GP) and mountain Grana Padano (M-GP) obtained from spring and summer milk. Fatty acids are expressed as g/100g of total fatty acids and CLA levels in mg/g fat.

No. analyzed samples	LH-GP						M-GP						Main effects (P<)			
	Spring			Summer			Spring			Summer						
	Lsmean	SE	137	Lsmean	SE	116	Lsmean	SE	25 of which 18 TG	Lsmean	SE	18 of which 10 TG				
Fatty acids (g/100g FA)	Lsmean	SE		Lsmean	SE		Lsmean	SE		Lsmean	SE		Season	Area	Season x area	
Trans11-18:1 (vaccenic acid, TVA)	0.94 ^A	0.02		0.95 ^A	0.03		1.19 ^B	0.06		1.66 ^C	0.07		***	***	***	
cis9-18:1 (oleic acid)	18.56 ^B	0.06		19.54 ^D	0.07		17.30 ^A	0.15		18.99 ^C	0.17		***	***	**	
18:2 (linoleic acid, LA)	2.35 ^C	0.02		2.33 ^{BC}	0.02		2.07 ^A	0.05		2.21 ^{AB}	0.06		ns	***	0.066	
18:3 omega3 (linolenic acid, LNA)	0.39 ^A	0.01		0.40 ^A	0.01		0.58 ^B	0.02		0.61 ^B	0.02		ns	***	ns	
Cis9, trans11 CLA (mg/g fat)	5.29 ^A	0.13		5.75 ^B	0.14		6.52 ^C	0.30		9.47 ^D	0.35		***	***	***	
Categories of fatty acids (g/100g FA):																
Short Chain Fatty Acids (SCFA) ¶	7.24 ^B	0.02		7.05 ^A	0.02		7.43 ^C	0.05		7.24 ^B	0.06		***	***	ns	
Medium Chain Fatty Acids (MCFA) ¶	21.23 ^C	0.09		19.82 ^A	0.10		22.72 ^D	0.22		20.64 ^B	0.25		***	***	*	
Long Chain Fatty Acids (LCFA) ¶	66.83 ^B	0.10		67.74 ^C	0.11		65.41 ^A	0.23		66.82 ^B	0.27		***	***	ns	
Saturated Fatty Acids (SFA)	65.59 ^B	0.11		64.42 ^A	0.12		67.42 ^C	0.25		64.23 ^A	0.29		***	***	***	
Monounsaturated Fatty Acids (MUFA)	25.70 ^B	0.08		26.19 ^C	0.09		24.17 ^A	0.19		26.08 ^{BC}	0.23		***	***	***	
Polyunsaturated Fatty Acids (PUFA)	4.01 ^A	0.03		4.00 ^A	0.03		3.97 ^A	0.07		4.38 ^B	0.08		***	**	***	

SE=Standard Error.

Season=production season of milk used in Grana Padano manufacturing (spring or summer); area=production area of Grana Padano cheese (lowland-hill or mountain); season x area=first order interaction.

LH-GP= Grana Padano cheese obtained from dairies placed in lowland-hill (altitude=0-500 m).

M-GP=Grana Padano cheese obtained from dairies placed in mountain (altitude >500 m).

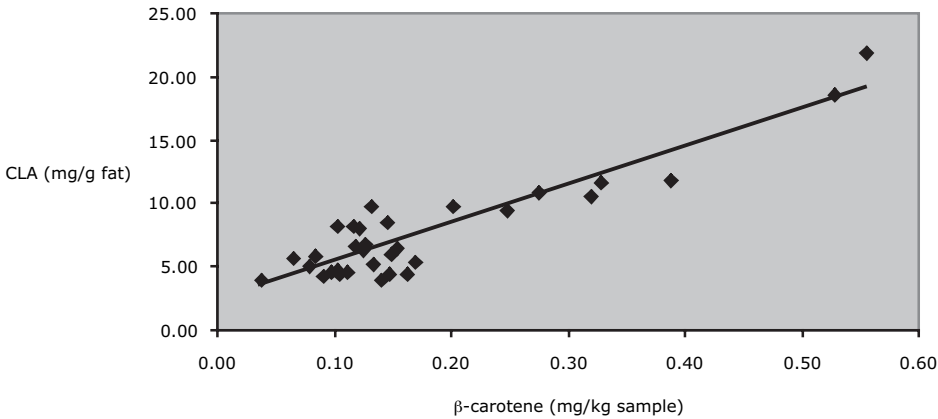
TG=Grana Padano cheese produced in the province of Trento.

¶ SCFA (C 4:0-C 9:0); MCFA (C 10:0-C 15:1); LCFA (C 16:0-C 22:6 omega3).

Different letters in the same line correspond to statistically significant differences (P<0.05); ns (not significant) refers to P>0.05.

*P<0.05; **P<0.01; ***P<0.001.

Figure 1. Correlation between β -carotene concentration and CLA content in summer Grana Padano.



in the animal's diet. With regard to this, it is important to underline that the majority of the samples collected from mountain dairies were Trentingrana cheese. This Grana Padano P.D.O. is produced in the province of Trento where animals are bred in the mountains (altitude > 800 m). Its Production Disciplinary, unlike that of Grana Padano, prohibits the use of silage in cow feed. During the conservation process, PUFA in herbage, which are precursors for the CLA formation in milk fat, may be reduced. Elgersma *et al.* (2003) reported a decrease in the proportion of LNA in ensiled material in comparison to fresh grass. LNA is a lipid substrate for the formation of TVA in the rumen and its subsequent desaturation to *cis-9,trans-11* CLA in the mammary gland (Bauman *et al.*, 2003). The greater CLA levels found in M-GP were in accordance with our previous surveys on the content of CLA in various milk products, where we reported high CLA levels in Fontina Valdostana (cheese produced from Valdostana raw cows' milk bred in the highest Europe alpine pasture land), Swiss Emmenthal (cow cheese obtained from animals kept on mountain pasture) and fermented milk

and yoghurt of mountain pasture (Prandini *et al.*, 2001, 2007).

Differences of fatty acid composition and CLA concentration were also seen between spring and summer both in LH-GP and M-GP, and with major entity in this last, displaying effects of the production season of the milk used in manufacturing and effects of interaction between production season and area. These findings could also be attributable to the animals' diet. Summer M-GP had a better fatty acid composition than spring M-GP, with higher levels of beneficial fatty acids for human health (CLA, TVA, oleic acid, MUFA and PUFA) and lower levels of detrimental fatty acids (SFA). The temperatures in spring in Italy's mountains are still cold so cows are fed a diet mainly based on hays and concentrates, whereas in the summer months with vegetative resumption, cows are mostly fed on pasture. A number of studies have confirmed that pasture feeding can increase the milk fat CLA concentrations in lactating dairy cows when they change from indoor winter feeding and that milk fat content increases with increasing proportions of pasture in the diet

(Harfoot and Hazlewood, 1997; Stanton *et al.*, 1997; Kelly *et al.*, 1998; Dhiman *et al.*, 1999). Moreover, a higher CLA content of milk could be due to different cow breeds; Kelsey *et al.* (2003) showed that Holstein, breed of lowland-hill has greater CLA content of milk compared to Brown Swiss in similar feeding condition.

The positive correlation found between β -carotene concentration and CLA content in summer Grana Padano suggests the presence of higher β -carotene concentrations in feed richer in CLA precursor fatty acids and vice versa. The high β -carotene (precursor of vitamin A) concentrations found in the summer M-GP improve the nutritional quality of this food. Milk fat represents a good dietary source of β -carotene (Panfili *et al.*, 1994), which exerts its antioxidant activity in biological tissues as well as in foods scavenging both singlet oxygen and lipoperoxides and thus preventing or limiting the oxidation of fatty acids (Donnelly and Robinson, 1995). The highest levels of CLA and β -carotene found in the organic sample produced in the lowland of the Verona province were in accordance with Bergamo *et al.* (2003). They in their study on fatty acid composition and fat-soluble vitamin concentrations in organic and conventional milk and dairy products found higher CLA, TVA, LNA, TH (α -tocopherol) and β -carotene contents in organic dairy foods. They reported that an organic diet, containing at least 60% dry matter of roughage, fresh or dried fodder (EC Reg. 2092/91 and 1804/99), may well improve microbial biohydrogenation, yielding higher levels of CLA in milk. Further, they suggested that fibre-rich organic diets may improve fat-soluble vitamin concentration in milk by decreasing milk yield.

In the lowland and hill areas of Italy cows are bred on farms and at least 50% of the dry matter of their daily diet is fresh or dried fodder. Today, permanent meadow

hays in some areas of lowland are being farmed alongside rotational crops, including alfalfa, which is considered to be one of the best fodders available. Over the years, summer and autumn cover crops have been added, and today corn silage is one of the most common crops grown for animal fodder throughout the Pianura Padana. In the lowland areas a large proportion of farms feed dairy cattle total mixed rations (unifed), the components of which must also comply with the Production Disciplinary issued by the *Consorzio per la Tutela del Formaggio Grana Padano* (2009).

A greater quantity of fresh forage is given to cows during the summer months, as a consequence of which, our study showed, in LH-GP there are higher levels of CLA, oleic acid and MUFA and lower levels of SFA compared to spring LH-GP. Moreover, hays fed during summer could have higher content of CLA precursors than hays fed in winter-early spring, due to the lower oxidation process related to the duration of the storage (Dewhurst *et al.*, 2006). Couvreur *et al.* (2006), in their work on the effect of replacing corn silage with increasing proportions of fresh cut grass in the cow diet on milk characteristics and on the functional and sensory properties of butter, found a linear increase in CLA, MUFA and PUFA at the expense of SFA percentage.

Conclusions

The fatty acid composition and CLA content of Grana Padano cheese were affected by the season and production area, and by the interaction of these two factors. In general, mountain Grana Padano reported a better fatty acid composition than lowland-hill Grana Padano, and in both improved fatty acid compositions were seen from spring to summer. Nevertheless, summer mountain Grana Padano showed the greatest values of CLA,

TVA, LNA and PUFA. Moreover, the β -carotene concentration was correlated to the CLA content suggesting the presence of higher β -carotene concentrations in feeds richer in CLA precursor fatty acids and vice versa.

Our results agree with those of previous studies on CLA concentration in milk and dairy products, and underline the important role of mountain cheese obtained from sum-

mer milk as a natural source of CLA and beneficial fatty acids for human health.

Financial support for this research from the Ministry of University and Research, Italy, is gratefully acknowledged (FISR project 2006-2008).

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