SHORT COMMUNICATION

Contents of conjugated linoleic acid isomers *cis9,trans11* and *trans10,cis12* in ruminant and non-ruminant meats available in the Italian market

Francesca M. Cicognini, Filippo Rossi, Samantha Sigolo, Antonio Gallo, Aldo Prandini

Istituto di Scienze degli Alimenti e della Nutrizione, Università Cattolica del Sacro Cuore, Piacenza, Italy

Abstract

Conjugated linoleic acid (CLA) isomers are considered healthy factors due to their anticarcinogenic, anti-atherosclerotic and lipolytic effect. A recommended daily intake from 0.8 to 3 g CLA/day/person has been proposed to obtain biological effects in humans. The aim of this work was to provide data on *cis9,trans11* (*c9,t11* CLA) and *trans10,cis12* (*t10,c12* CLA) contents in meats collected from Italian large-scale retail trade and completing a food CLA database. In a first trial, beef loin meats were characterised for label information available for consumers: origin (*i.e.*, Ireland, France-Italy, Piedmont) and sex of animals. No differences were observed for *c9,t11* and *t10,c12* CLA contents (mg/g fat) of loin meat from male or female. Piedmontese meat showed lower ($P<0.05$) *c9,t11* CLA level (mg/g fat) than Irish and French-Italian meats, whereas similar *t10,c12* CLA contents were measured in Piedmontese, Irish and French-Italian meats. Successively, meat samples from different animal species (male and female beef, veal, suckling lamb, belly beef, canned beef meat, pork and horse) were characterised for their contents in *c9,t11* and *t10,c12* CLA. Lamb meat had the highest ($P<0.05$) *c9,t11* CLA content (mg/g fat). The *c9,t11* CLA was lower than 2 mg/g fat in veal, pork and horse meats. Low *t10,c12* CLA amounts were found in all analysed meat samples. These data provided information to estimate the average daily intake of CLA from meats in an Italian cohort, which can be used in epidemiological studies.

Introduction

The conjugated linoleic acid (CLA) importance is related mainly to the healthy properties of two isomers, *cis9,trans11* CLA (*c9,t11* CLA) and *trans10,cis12* CLA (*t10,c12* CLA) (Bhattacharya *et al*., 2006; Stringer *et al*., 2010). Anticancer effects have been attributed to *c9,t11* CLA (Bhattacharya *et al*., 2006). The *t10,c12* CLA has been reported as CLA isomer responsible for improving features of the metabolic syndrome (Stringer *et al*., 2010). Conjugated linoleic acid supplementation has been recommended of 0.8 to 3 g per day based on anticancer effects of CLA (Ip *et al*., 1994; Parish *et al*., 2003).

Food sources derived from ruminants are significantly richer in CLA than those from monogastric animals (Schmid *et al*., 2006). As a matter of fact, CLA is produced by either ruminal biohydrogenation of dietary linoleic and linolenic acids or by endogenous synthesis from trans-vaccenic acid via Δ^9-desaturase (Griinari and Bauman, 1999; Griinari *et al*., 2000).

The aims of this work were to determine the contents of *c9,t11* and *t10,c12* CLA isomers in beef loin meats as related to sex and origin of animals, based on meat label information, and to assess the contents of these CLA isomers in meats from different animal species (ruminant and non-ruminant). The current study represented an integrative part of an extended work conducted for estimating the *c9,t11* and *t10,c12* CLA contents in foods (Prandini *et al*., 2001, 2007, 2009a, 2009b, 2011; Cicognini *et al*., 2014).

Materials and methods

Sampling

The meat samples were purchased in prewrapped food trays during a one-year period (from January to December 2011) at the most spread and with the highest turnover rate large-scale retail trade (LRT) brand in Italy (FederDistribuzione, 2013). Male and female heifer beef loin meats (total of 42 samples) of three different origins (Piedmont, Ireland and French-Italy) were collected (T trial). Moreover, a total of 84 samples of ruminant and non-ruminant meats were collected including male and female beef, suckling lamb, belly beef, canned beef meat, veal, pork and horse (S survey).

Corresponding author: Dr. Francesca Maria Cicognini, Istituto di Scienze degli Alimenti e della Nutrizione, Università Cattolica del Sacro Cuore, via Emilia Parmense 84, 29122 Piacenza, Italy.
Tel. +39.0523.599286 - Fax: +39.0523.599259.
E-mail: francescamaria.cicognini@unicatt.it

Key words: *cis9,trans11* CLA, *trans10,cis12* CLA, Meat, Italian market, Animal origin.

Acknowledgements: this research was supported by a grant from Fondazione Romeo e Enrica Invernizzi, Milano, Italy.

Received for publication: 29 September 2013.
Accepted for publication: 17 January 2014.

Italian Journal of Animal Science 2014; 13:3125
doi:10.4081/ijas.2014.3125

Chemical analysis

Fat content was determined in accordance with the UNI ISO method 1443:1991 through a Soxhlet extraction after acid digestion (ISO, 1991).

The lipid extraction was performed according to Folch's technique (Christie, 1989) as modified by Prandini *et al*. (2007). The preparation of *c9,t11* and *t10,c12* CLA methyl esters was conducted in accordance with the method described by Prandini *et al*. (2007). GC instrument, oven parameters and gas variables for *c9,t11* and *t10,c12* CLA methyl esters quantification were as previously described (Cicognini *et al*., 2014). The limit of detection (LOD) was 0.005 mg/g fat, while the limit of quantification (LOQ) was 0.01 mg/g fat. The *c9,t11* and *t10,c12* isomer peaks were identified by comparison with the retention times of reference standards (methyl *c9,t11* and *t10,c12* octadecadienoate; Matreya, Pleasant Gap, PA, USA). Since a peak overlapping was found for C 19:0 [internal standard (IS); nonadecanoate methyl ester acid; Sigma-Aldrich, St. Louis, MO, USA], the area of interfering peak was measured running each sample twice, with and without IS. In our study, the co-elution of the *c9,t11+t7,c9+t8,c10* triplet (Blasko *et al*., 2009) was not checked owing to commercial unavailability of the *t7,c9* and *t8,c10* isomer standards. Nevertheless, these isomers occur in low amounts in ruminant fat (Yurawecz *et al.*, 1998; Fritsche *et al.*, 2000; Serra *et al.*, 2009).

Statistical analysis

Data were tested for normality with the Shapiro-Wilk test. Non-normal variables were log-normal transformed before statistical analysis (Petrie and Watson, 2006) and Levene's test was carried out to verify equality of variances across each combination of two tested factors in T trial (*i.e.*, sex and origin) and meat types in S survey. When variances were homogenous, data were analysed by Mixed procedure of SAS (2010) by using respectively two- or one-way analyses of variance (ANOVA) with equal variances in T trial or S Survey, otherwise ANOVA with unequal variances was used. In this case, the Welch's statistic was used to provide a test of equality of means across the levels of tested factors. Mean *post-hoc* comparisons were assessed with the LSMEANS statement and the respective level of significance was adjusted according to the Tukey-Kramer method (Littell *et al.*, 2006). The significance level was set at P<0.05.

Results and discussion

T trial

Table 1 shows the *c9,t11*, *t10,c12* CLA and fat contents of beef loin meats. No differences were found in the *c9,t11* and *t10,c12* CLA contents (mg/g fat) of loin meat from male and female animals. Female loin meat was 58% richer (P<0.05) in fat than male loin meat, consequently female meat contained an amount of *c9,t11* CLA (mg/100 g meat) about two-times higher (P<0.05).

Piedmontese meat showed lower (P<0.05) content of intramuscular fat (-68% on average) compared with Irish and French-Italian meats. The Piedmontese breed is characterised by muscle hypertrophy or double-muscled. In agreement with our result, this characteristic was associated with meat with lower fat content than meat from normal animals (Aldai *et al.*, 2006). Piedmontese meat exhibited also lower (-35% on average; P<0.05) *c9,t11* CLA level (mg/g fat) than Irish and French-Italian ones. When data were expressed as mg/100 g meat the highest and lowest (P<0.05) values were 29.36 for French-Italy and 4.36 in Piedmont, respectively. Differences in fat content and a possible lower activity of Δ^9 desaturase enzyme in leaner animals, as reported by Aldai *et al.* (2006) and Brugiapaglia *et al.* (2013), could explain the lower *c9,t11* CLA content in leaner animals compared with fatter animals. In agreement with Brugiapaglia *et al.* (2013), a positive correlation between *c9,t11* CLA and fat content was observed in the current experiment (P<0.05, r=0.25). No difference due to origin of meats was instead observed in *t10,c12* CLA content. On the other hand, the ruminal biohydrogenation is the only synthesis pathway responsible for the *t10,c12* CLA level in ruminant products as animal tissues do not possess the desaturase enzyme capable of inserting a C 12-double bond into the *t*10 C18:1 molecule (Raes *et al.*, 2004).

S survey

Table 2 shows the *c9,t11*, *t10,c12* CLA and fat contents of meats from different animal species. Suckling lamb meat had the highest (P<0.05) *c9,t11* CLA content (mg/g fat). In agreement, a review of Schmid *et al.* (2006)

Table 1. Average *c9,t11, t10,c12* CLA and fat contents of beef loin meat from animals of different sex and origin.

	n	*c9,t11* CLA°, mg/g fat	*t10,c12* CLA#, mg/g fat	*c9,t11* CLA°, mg/100 g meat	*t10,c12* CLA#, mg/100 g meat	Fat#, %
Sex§						
Male^	18	2.98	0.04	8.23^{b}	0.10	2.54^{b}
Female	19	2.88	0.01	17.76^{a}	0.06	5.98^{a}
Origin§						
Ireland	9	3.34^{a}	0.01	13.27^{ab}	0.05	4.46^{a}
France-Italy	14	3.40^{a}	0.03	29.36^{a}	0.16	6.70^{a}
Piedmont	14	2.19^{b}	0.04	4.36^{b}	0.04	1.80^{b}
√MSE		1.240	1.091	14.25	1.091	0.687
P						
Sex		ns	ns	<0.05	ns	<0.05
Origin		<0.05	ns	<0.05	ns	<0.05

MSE, mean-square error. °Interaction of sex and origin was not statistically significant; #*c9,t11* CLA is referred to *c9,t11+t7,c9+t8,c10* triplet; §values were log-normal transformed before statistical analysis; ^all the meat samples were obtained from bulls. ^{a,b}Means in the same column within sex and origin with different superscripts are significantly different (P<0.05).

Table 2. *c9,t11, t10,c12* CLA and fat contents of meats from animals of different species.

Meat type	n. (total=84)	*c9,t11* CLA°, mg/g fat	*t10,c12* CLA#, mg/g fat	*c9,t11* CLA°#, mg/100 g meat	*t10,c12* CLA#, mg/100 g meat	Fat#, %
Ruminant meat						
Beef male	18	2.98^{bc} (1.53)	0.04^{b} (0.07)	8.41^{bc} (7.83)	0.12^{bc} (0.22)	2.54^{c} (1.87)
Beef female	19	2.88^{bc} (1.25)	0.01^{b} (0.01)	17.59^{b} (10.36)	0.06^{bc} (0.05)	5.98^{bc} (5.17)
Veal	15	1.28^{c} (0.52)	0.07^{ab} (0.07)	6.82^{bc} (6.10)	0.39^{b} (0.37)	5.15^{bc} (4.14)
Suckling lamb	6	9.84^{a} (3.37)	0.02^{b} (0.02)	103.96^{a} (63.62)	0.34^{b} (0.31)	11.59^{ab} (6.93)
Belly beef	6	4.24^{b} (1.39)	0.36^{a} (0.28)	75.13^{a} (39.64)	9.27^{a} (10.92)	19.81^{a} (11.83)
Canned beef meat	7	2.01^{bc} (0.94)	0.01^{b} (0.00)	2.62^{cd} (1.23)	0.01^{c} (0.00)	1.31^{c} (0.16)
Non-ruminant meat						
Pork	7	0.67^{c} (0.33)	0.01^{b} (0.01)	3.45^{cd} (3.3)	0.08^{bc} (0.08)	4.80^{bc}±3.04
Horse	6	0.34^{c} (0.17)	0.01^{b} (0.00)	0.91^{d} (0.75)	0.03^{c} (0.01)	2.73^{c}±1.48
√MSE^{2}		1.747	0.822	0.976	1.320	0.810
P		<0.05	<0.05	<0.05	<0.05	<0.05

MSE, mean-square error. °*c9,t11* CLA is referred to *c9,t11+t7,c9+t8,c10* triplet; #values were log-normal transformed before statistical analysis. Values are expressed as means and standard deviation (in brackets). $^{a-d}$Means in the same column with different superscripts are significantly different (P<0.05).

reported that *c9,t11* CLA content in lamb meat can range from 4.3 to 19.0 mg/g fat and this could be associated with diet, being sheep milk rich in CLA (Contarini *et al*., 2009). According to their high fat content, suckling lamb meat and belly beef showed the highest (P<0.05) *c9,t11* CLA amount as mg/100 g meat.

The *c9,t11* CLA was lower than 2 mg/g fat in veal, pork and horse meats. The *c9,t11* CLA amount in veal meat depends on the diet mainly based on cows' milk derivatives. Being non-ruminant animals, pork and horse meats showed *c9,t11* CLA levels lower than 1 mg/g fat, in agreement with previous studies (Schmid *et al*., 2006). Studies carried out on mice have shown that CLA is synthetised endogenously from dietary vaccenic acid (C18:1 t11) (Khanal and Dhiman, 2004). Synthesis of CLA from trans-vaccenic acid has been shown to occur in humans too (Adolf *et al*., 2000). Moreover, Alonso *et al*. (2003) reported that several species of bacteria derived from the human intestine could synthesise CLA.

Amounts of *t10,c12* CLA lower than instrumental LOQ (0.01 mg/g fat) were found in several analysed meat samples. Belly beef showed the highest (P<0.05) level of *t10,c12* CLA both as mg/g fat and mg/100 g meat.

Conclusions

This study provided information on the *c9,t11* and *t10,c12* CLA contents in meats available in the Italian LRT. Generally, meat contained low amounts of *c9,t11* CLA and almost negligible levels of *t10,c12* CLA for appreciation of health benefits in humans. Consequently, the consumption of other food, such as milk and their by-products, should be encouraged to improve daily CLA intake by humans. Alternatively, specific feeding strategies should be taken into account in order to enhance the *c9,t11* and *t10,c12* CLA contents in meat.

References

Adolf, R., Duval, S., Emeken, E., 2000. Biosynthesis of conjugated linoleic acid in humans. Lipids 35:131-135.

Aldai, N., Murray, B.E., Oliván, M., Martínez, A., Troy, D.J., Osoro, K., Nájera, A.I., 2006. The influence of breed and mh-genotype on carcass conformation, meat physico-chemical caracteristics, and the fatty acid profile of muscle from yearling bulls. Meat Sci. 72:486-495.

Alonso, L., Cuesta, E.P., Gilliand, S.E., 2003. Production of free conjugated linoleic acid by Lactobacillus acidophilus and Lactobacillus casei of human intestinal origin. J. Dairy Sci. 86:1941-1946.

Bhattacharya, A., Banu, J., Rahaman, M., Causey, J., Fernandes, G., 2006. Biological effects of conjugated linoleic acids in health and disease. J. Nutr. Biochem. 17:789-810.

Blasko, J., Kubinec, R., Ostrovsky, I., Pavlikova, E., Krupcik, J., Sojak, L., 2009. Chemometric deconvolution of gas chromatographic unresolved conjugated linoleic acid isomers triplet in milk samples. J. Chromatogr. A 1216:2757-2761.

Brugiapaglia, A., Lussiana, C., Destefanis, G., 2013. Fatty acid profile and cholesterol content of beef at retail of Piemontese, Limousin and Friesian breeds. Meat Sci. 96:568-573.

Christie, W.W., 1989. Gas chromatography and lipids: a pratical guide. The Oily Press, Dundee, UK.

Cicognini, F.M., Rossi, F., Sigolo, S., Gallo, A., Prandini, A., 2014. Conjugated linoleic acid isomer (cis9,trans11 and trans10,cis12) content in cheeses from Italian large-scale retail trade. Int. Dairy J. 34:180-183.

Contarini, G., Pelizzola, V., Povolo, M., 2009. Content of conjugated linoleic acid in neutral and polar lipid fractions of milk of different ruminant species. Int. Dairy J. 19:342-344.

FederDistribuzione, 2013. Mappa del sistema distributivo italiano. Available from: http://www.federdistribuzione.it/studi_ricerche/files/Mappa_Distributiva.pdf

Fritsche, J., Fritsche, S., Solomon, M.B., Mossoba, M.M., Yurawecz, M.P., Morehouse, K., Ku, Y., 2000. Quantitative determination of conjugated linoleic acid isomers in beef fat. Eur. J. Lipid Sci. Tech. 102:667-672.

Griinari, J.M., Bauman, D.E., 1999. Biosynthesis of conjugated linoleic acid and its incorporation into meat and milk in ruminants. In: M. Yurawecz, M.M. Mossoba, J.K.G. Kramer, M.W. Pariza and J.G. Nelson (eds.) Advances in conjugated linoleic acid research. AOCS Press, Champaign, IL, USA, pp 180-200.

Griinari, J.M., Corl, B.A., Lacy, C.A., Chouinard, P.Y., Nurmela, K.V.V., Bauman, D.E., 2000. Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by D9-desaturase. J. Nutr. 130:2285-2291.

Ip, C., Singh, M., Thompson, H.J., Scimeca, J.A., 1994. Conjugated linoleic acid suppresses mammary carcinogenesis and proliferative activity of the mammary gland in the rat. Cancer Res. 54:1212-1215.

ISO, 1991. Meat and meat products. Determination of total fat content. Norm UNI ISO 1443:1991. International Organization for Standardization, Geneva, Switzerland.

Khanal, R.C., Dhiman, T.R., 2004. Biosynthesis of conjugated linoleic acid (CLA): a review. Pak. J. Nutr. 3:72-81.

Littell, R.C., Milliken, G.A., Stroup, W.W., Wolfinger, R.D., Schabenberger, O., 2006. SAS for Mixed Models, Second Edition. SAS Institute Inc., Cary, NC, USA.

Parish Jr., F.C., Wiegand, B.R., Beitz, D.C., Ahn, D.U., Du, M., Trenkle, A.H., 2003. Use of dietary CLA to improve composition and quality of animal-derived foods. In: J.L. Sebedio, W.W. Christie and R. Adlof (eds.) Advances in conjugated linoleic acid research. AOCS Press, Champaign, IL, USA, pp 189-217.

Petrie, A., Watson, P.F., 2006. Statistics for veterinary and animal science. Blackwell Publishing Ltd., Oxford, UK.

Prandini, A., Geromin, D., Conti, F., Masoero, F., Piva, A., Piva, G., 2001. Survey on the level of conjugated linoleic acid in dairy products. Ital. J. Food Sci. 13:243-253.

Prandini, A., Sigolo, S., Cerioli, C., Piva, G., 2009a. Survey on conjugated linoleic acid (CLA) content and fatty acid composition of Grana Padano cheese produced in different seasons and areas. Ital. J. Anim. Sci. 8:531-540.

Prandini, A., Sigolo, S., Piva, G., 2009b. Conjugated linoleic acid (CLA) and fatty acid composition of milk, curd and Grana Padano cheese in conventional and organic farming systems. J. Dairy Res. 76:278-282.

Prandini, A., Sigolo, S., Piva, G., 2011. A comparative study of fatty acid composition and CLA concentration in commercial cheeses. J. Food Compos. Anal. 24:55-61.

Prandini, A., Sigolo, S., Tansini, G., Brogna, N., Piva,G., 2007. Different level of conjugated linoleic acid (CLA) in dairy products from Italy. J. Food Compos. Anal. 20:472-479.

Raes, K., De Smet, S., Demeyer, D., 2004. Effect of dietary fatty acids on incorporation of long chain polyunsaturated fatty acids and conjugated linoleic acid in lamb, beef and pork meat: a review. Anim. Feed Sci. Tech. 113:199-221.

Schmid, A., Collomb, M., Sieber, R., Bee, G., 2006. Conjugated linoleic acid in meat and meat products: a review. Meat Sci. 73:29-41.

Serra, A., Mele, M., La Comba, F., Conte, G., Buccioni, A., Secchiari, P., 2009. Conjugated Linoleic Acid (CLA) content of meat from three muscles of Massese suckling lambs slaughtered at different weights. Meat Sci. 81:396-404.

SAS, 2010. SAS/SAT guide for personal computers, version 9.3. SAS Inst. Inc., Cary, NC, USA.

Stringer, D.M., Zahradka, P., DeClercq, V.C., Ryz, N.R., Diakiw, R., Burr, L.L., Xie, X., Taylor, C.G., 2010. Modulation of lipid droplet size and lipid droplet proteins by trans-10, cis-12 conjugated linoleic acid parallels improvements in hepatic steatosis in obese, insulin-resistant rats. Biochim. Biophys. Acta 1801:1375-1385.

Yurawecz, M.P., Roach, J.A.G., Sehat, N., Mossoba, M.M., Kramer, J.K.G., Fritsche, J., Steinhart, H., Ku, Y., 1998. A new conjugated linoleic acid isomer, 7 trans, 9 cis-octadecadienoic acid, in cow milk, cheese, beef and human milk and adipose tissue. Lipids 33:803-809.