Increase of Conjugated Linoleic Acid Content of Dairy Food by Feeding

Éva VARGA-VISI
János CSAPÓ

SUMMARY

Conjugated linoleic acids (CLA) belong to the family of recently discovered anti-cancer factors found in milk fat. In order to exert a significant protection against the development of cancer, intake of CLA should be increased, which can be accomplished with the consumption of food products with enhanced CLA level. The increase of CLA content in milk fat can be obtained through feeding by modification of the biological hydrogenation processes in the rumen. The factors which exert an effect on the amount of CLA formed in conjunction with feeding are the amount and composition of the oil supplementation, the stage of the fat in the diet (e.g. free fat addition or fat within seeds), the structure of the fat-carrier, the fiber content and energy level of feed and the feeding regimen. While assembling diets the above factors should be applied in such a manner that paralell with the increase of CLA content of milk fat possible negative effects due to emerged oil consumption e.g. milk fat depression, decrease of the digestibility of nutrients and that of feed intake would be minimized.

KEY WORDS

conjugated linoleic acids, biological hydrogenation, milk fat, oil supplementation

University of Kaposvár, Faculty of Animal Science
Guba S. u. 40, H-7400 Kaposvár, H-7400, Hungary
Received: June 23, 2003

INTRODUCTION

Milk fat recently was considered unamously to be harmful for human health due to its high saturated fatty acid content. Nowadays results of new researches pointed out that it contains components which may exert positive effects on health. Conjugated linoleic acids (CLA) have been shown to inhibit the development of skin and stomach cancer in mice (Pariza and Hargraves, 1985; Ha et al., 1987, 1990), and that of breath cancer in rats (Ip et al., 1991, 1994), and exert a cytotoxic effect on the cell cultures of human tumor cells (Schultz et al., 1992). After discovering these advantegous effects of CLA, different sort of foods were investigated in order to determine, which can be the best source of CLA. Animal product of ruminants (dairy products and meat) were found to contain the highest amount of CLA (0,2 - 2 g CLA/ 100 g fat) while products of monogastric animals contain about one order of magnitude less CLA than that of ruminants (Ha et al., 1989; Chin et al., 1992a, 1992b, Lin et al., 1995, Frtische and Steinhart, 1998; Jiang et al., 1996). In vegetal fat only traces of CLA occur (Fritsche and Steinhart, 1998). Based on the results of experiments with animals, the daily intake dosis of CLA, which can effectively contribute to the prevention of cancer in humans - 3,5 g CLA/day - (Ip et al., 1991) is significantly higher than the average true CLA consumption (0,5- 1,5 g/day) (Parodi, 1994; Fritsche and Steinhart, 1998). With the increase of consumption of commercial dairy product the effective intake level cannot be achieved e.g. it may mean drinking 20-30 litre of milk/day (Jiang et al. 1998). The solution could be the production of food with enchanced CLA-level. There are three different approaches to reach this aim. The first one is synthetic CLA addition to the food product (Fritsche and Steinhart, 1998, Garcia et al., 2000). The second is addition of meat fat fraction enriched in CLA with extraction to the food (Romero at. al, 2000). And at last CLA content of dairy products can be increased with different feeding techniques (Jiang et.al, 1996; Precht and Molkentin, 2000; Dhiman et al., 2000; Donovan et al., 2000; Bauman et al., 2000).

DEFINITION AND BIOLOGICAL FORMATION OF CLA

CLA is a complex mixture of different positional isomers of linoleic acid (cis-9,cis-12–C18:2). The two conjugated double-bonds occur most frequently at the 9,11 or at the 10,12 position (Ha et al., 1987), but can also can be between other carbons that is 8,11; or 11,13 with both of cis and trans configuration.

The highest part of CLA found in animal products form during the biological hydrogenation of poliunsaturated fatty acids (PUFA) in the digestive track (Shorland et al., 1955; Chin et al., 1992a). Production of CLA in the intestine of monogastric animals cannot be excluded but has minor significance related to ruminants. Another synthetic pathway is also possible in the mammary gland of cows (Griinari and Bauman, 1999) or in the liver of rats (Pollard et al., 1980; Holman and Mahfouz, 1981) from trans-11-C18:1 (vaccenic acid) via Δ^9-desaturase (Griinari et al., 2000). The most frequently occurring CLA-isomer in biological products is cis-9,trans-11-C18:2 (c9,t11-CLA) (Kepler and Tove, 1967a). The formation of c9,t11-CLA has been proposed to occur in the first step of biohydrogenation of linoleic acid (cis-9,cis-12-C18:2). This intermediate is then hydrogenated to form a mixture of mainly trans-vaccenic acid (trans-11-C18:1) and elaidic acid (trans-9-C18:1). These monosaturated fatty acids can be further hydrogenated to saturated stearic acid (C18:0) (Kepler et al., 1971). The strong linear correlation between the concentration of c9,t11-CLA and t11-C18:1 suggested that the first two steps in the biohydrogenation reaction (from c9,c12-C18:2 via c9,t11-C18:2 to t11-C18:1) were not rate-limiting (Jiang at al., 1996). These two reactions were catalyzed by enzymes from one of the distinct populations of hydrogenation microorganisms in the rumen, i.e. Butyrivibrio fibrisolvens (Kepler és Tove, 1967b), but the hydrogenated step of trans-11-C18:1 to C18:0 was not related to activity by those bacteria, and was the rate-limiting step in the complete biohydrogenation of linoleic acid to stearic acid (Kemp et al., 1975; Polan et al, 1964).

INCREASE OF CLA IN THE PRODUCTS OF RUMINANTS BY FEEDING

The factors related to feeding which exert an effect on CLA content of milk fat are the unsaturated fatty acid (principally linoleic and linolenic acid) content of feed, the stage of fat in the diet (free fat addition or fat within seeds), the structure of the fat-carrier, the fiber content and the energy level of feed, and the feeding frequency. The influence of some of the other factors such as different seasons or production systems can be attributed to the effect of disparity in feeding conditions.

A long time ago it was observed, when cattle was driven out to pasture in spring that the absorption of milk fat at ultraviolet range increased (Booth and Kon, 1935). In fact, the amount of CLA was detected with this measurement due to the absorption of conjugated diene system at 230 nm. After the development of spectroscopic methods for detection, CLA content of raw milk was detected to be significantly higher in summer than in winter (Riel, 1963). Nowadays the same seasonal variation was observed (Wolff et al., 1995; Dhiman et al., 1996; Precht and Molkentin, 2000). The reason of this fluctuation in CLA content is probably the difference in diets. Cattle grazing pasture had higher CLA content in milk than in barn feeding with hay and silage (Dhiman et al., 1996; Precht and Molkentin, 2000). Pasture grasses are rich source of linolenic acid (C18:3). Because CLA is an intermediate of biohydrogenation of poliunsaturated fatty acids (PUFA), the amount of CLA formed in the rumen can be increased with diets rich in PUFA. When the dietary supply of PUFA is high the biohydrogenation process may be incomplete, the CLA can escape the rumen and become available for absorption in the lower digestive tract, thus providing a source of CLA to the mammary gland (Dhiman et al., 2000).

Feeding of oils rich in linoleic acid (C18:2) e.g. soybean, cottonseed, linseed and sunflower oil were also reported to be useful in increasing CLA content of milk (Kelly et al. 1998; Dhiman et al. 1999, 2000). Beyond the intake dosis and the composition of the oil supplementation, the structure of the oil source has also exerted an effect on the formation of CLA. The intake of raw cracked soybeans did not increase the level of CLA in milk of cattle but roasted cracked soybeans or soybean oil did. If soybeans are not treated by heat, the release of oil is slower than in case of roasted soybeans. Heat treatment makes soybeans brittle, and as a result, may increase the release of oil. If oil releases slowly from oil seeds in the rumen, accumulation of intermediates has relatively less probability than in case of free oil supplement, or oil encapsulated in a fragile structure.

The type of the oil source also exert an effect on CLA-level of milk fat. Soybean oil has been shown to be more effective than lineseed oil (Dhiman et al., 2000). Despite the fact that the linoleic acid content of fish oil is not high, this oil approved to be also an efficient oil source in increasing CLA, but the biochemical mechanism of this phenomenon has not yet been clarified (Harfoot and Hazelwood, 1988).

The effect of the oil supplementation on CLA content of milk fat depends also on the levels of addition. When soybean oil (at 0.5%, 1.0%, 2.0%, 4.0 %) or linseed oil (at 1.0% of the dietary dry matter) was used in order to partly replace the feed of cows, the CLA content of milk increased significantly related to control only at the two highest levels of soybean oil addition (Dhiman et al., 2000). In case of 2% fish oil supplement the CLA content of milk fat increased significantly but not in case of 1% level of addition (Donovan et al., 2000). The decrease of the digestibility of nutrients and that of feed intake limits the further increase of oil content of feed and therefore that of CLA in milk fat. Another fact is that increase of the free oil content of feed may result in depression of milk fat content (Jenkins, 1993). Therefore paralel with the increase of CLA in milk fat, the fat content of milk may decrease. This phenomenon can be avoided by using oil source with structure releasing oil in such a degree that significant CLA formation can be assured without significant milk fat depression e. g. roasted cracked soybeans (Dhiman et al., 2000).

Enhanced intake of free oils in ruminants besides milk fat depression may cause decrease in the protein content of milk (Grummer, 1988) due to the inhibition of rumen fermentation. Attention has been drown to the fact that this phenomenon does not occur if the protein supplementation in the small intestine derived from feed is adequate for the production level of cows (Dhiman et al., 2000).

Traits that influence rumen fermentation also exert an effect on biohydrogenation and therefore influence the CLA content of milk fat, e. g. grain: forage ratio. If the starch: fiber ratio is higher, the terminal step of biohydrogenation is slower (Gerson et al., 1985). High concentrate diets interfered with the terminal hydrogenation step and the amount of CLA (Jiang et al., 1996, 1998) and trans-11-C18:1 (Palmquist and Schanbacher, 1991) increases substantially in milk fat of cows fed high grain diets.

The number of feeding per day can also influence the biohydrogenation process. If ruminants intake their feed fewer times so they eat higher portion of feed at once, the amount of substrate for biohydrogenation is high and due to the rate-limiting step the amount of intermediate products e. g. CLA increase. Ad libitum feeding resulted in lower concentration of CLA in milk than did restricted amount of feed in portions (Jiang et al., 1996). The length of period while animals supplied with high oil content feed can also be an important factor. The average concentration of CLA in milk fat of cows declined after a few week of administration of these diets (Bauman et al., 2000) probably due to the changing in the biohydrogenation processes because of the adaptation of microbe population in the rumen.

The increase of CLA content in milk seems to be accomplished with adequate feeding techniques. However, other factors still unknown may exert an important effect on the formation of CLA and therefore CLA content of milk fat because in most studies huge diversity was observed among individuals in CLA forming ability.

REFERENCES

Bauman, D.E., Barbano, D.M., Dwyer, D.A., Griinari, J.M. (2000). Technical note: production of butter with enhanced conjugated linoleic acid for use in biomedical studies with animal models. J. Dairy Sci., 2000. 83. 2422-2425.

Booth, R.G., Kon, S.K. (1935). A study of seasonal variation in butter fat. J. Biochem., 29. 133-137.

Chin, S.F., Liu, W., Albright, K., Pariza, M.W. (1992a). Tissue levels of cis-9,trans-11 conjugated dienoic isomer of linoleic acid (CLA) in rats fed linoleic acid (LA). Faseb J., 6. A1396.

Chin, S.F., Liu, W., Storkson, J.M., Ha, Y.L., Pariza, M.W. (1992b). Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognised class of anticarcinogens. J. Food Comp. Anal., 5. 185-197.

Dhiman, T.R., Anand, G.R., Satter, L.D., Pariza, M.W. (1996). Dietary effects on conjugated linoleic acid content of cow's milk. 87th AOCS Annul Meeting and Expo, USA.

Dhiman, T.R., Helmink, E.D., McMahon, D.J., Fife, R.L., Pariza, M.W. (1999). Conjugated linoleic acid content of milk and cheese from cows fed extruded oilseeds. J. Dairy Sci., 82. 412-419.

Dhiman, T.R., Satter, L.D., Pariza, M.W., Galli, M.P., Albright, K., Tolosa, M.X. (2000). Conjugated linoleic acid (CLA) content of milk from cows offered diets in linoleic acid. J. Dairy Sci., 83. 1016-1027.

Donovan, D.C., Schingoethe, D.J., Baer, R.J., Ryali, J., Hippen, A.R., Franklin, S.T. (2000). Influence of dietary fish oil on conjugated linoleic acid and other fatty acids in milk fat from lactating dairy cows. J. Dairy Sci., 83. 2620-2628.

Fritsche, J., Steinhart, H. (1998). Amounts of conjugated linoleic acid (CLA) in German foods and evaluation of daily intake. Z. Lebensm Unters Forsch A, 206. 77-82.

Garcia, H.S., Keough, K.J., Arcos, J.A, Jr. Hill, G.C. (2000). Interesterification (acidolysis) of butterfat with conjugated linoleic acid in batch reactor. J. Dairy Sci., 83. 371-377.

Gerson, T., Jihn, A., King, A.S.D. (1985). The effect of dietary starch and fibre on the in vitro rates of lipolysis and hydrogenation by sheep rumen digesta. J. Agric. Sci., 105. 27.

Griinari, J., Bauman, T.B. (1999). Biosynthesis of conjugated linoleic acid and its incorporation into meat and milk in ruminants. Advances in Conjugated Linoleic acid Research. Eds. Yuracez, M.W., Mossoba, M.M., Kramer, J.K.G., Pariza, M.W., Nelson, G. AOCS Press, Champaign, IL, 1. 180-198.

Griinari, J., Corl, B.A., Lacy, P.Y., Chouinard, K.V., Nurmela, V., Bauman, D.E. (2000). Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by Δ^9-desaturase. J. Nutr., 130. 2285-2291.

Grummer, R.R. (1988). Influence of prilled fat and calcium salt of palm oil fatty acids on ruminal fermentation and nutrient digestibility. J. Dairy Sci., 71. 117-123.

Ha, Y.L., Grimm, N.K., Pariza, M.W. (1987). Anticarcinogens from fried ground beef: heat-altered derivatives of linoleic acid. Carcinogenesis, 8. 1881-1887.

Ha, Y.L., Grimm, N.K., Pariza, M.W. (1989). Newly recognized anticarcinogenic fatty acids: identification and quantification in natural and processed cheeses. J. Agriculture and Food Chemistry, 37. 75-81.

Ha, Y.L., Storrkson, J., Pariza, M.W. (1990). Inhibition of benzo(a)prene-inducted mouse forestomach neoplasis by conjugated dienoic derivatives of linoleic acid. Cancer Res., 50. 1097-1101.

Harfoot, C.G., Hazelwood, G.P. (1988). Lipid metabolism in the rumen. The Rumen Microbiological Ecosystem. Ed. Hobson, P.N., Elsevier Applied Sci. Publishers, London, 285-322.

Holman, R.T., Mahfouz, M.M. (1981). Cis- and trans-octadecenoic acids as precursors of polyunsaturated acids. Prog. Lipid Res., 20. 151-156.

Ip, C., Chin, S.F., Scimeca, J.A., Pariza, M.W. (1991). Mammary cancer prevention by conjugated dienoic derivative of linoleic acid. Cancer Res., 51. 6118-6124.

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.

Jenkins, T.C. (1993). Lipid metabolism in the rumen. J. Dairy Sci., 76. 3851-3863.

Jiang, J., Björck, L., Fondén, R. (1998). Production of conjugated linoleic acid by dairy starter cultures. Doctoral thesis.

Jiang, J., Björck, L., Fondén, R., Emanuelson, M. (1996). Occurrence of conjugated cis-9,trans-11-octadecadienoic acid in bovine milk: effects of feed and dietary regimen. J. Dairy Sci., 79. 438-445.

Kelly, M.L., Berry, D.A., Dwyer, J.M., Griinari, J.M., Chouinard, P.Y., Amburgh, M.E.W., Bauman, D.E. (1998). Dietary fatty acid sources affect conjugated linoleic acid concentrations in milk from lactating dairy cows. J. Nutr., 128. 881-885.

Kemp, P., White, R.W., Lander, D.J. (1975). The hydrogenation of unsaturated fatty acids by five bacterial isolates from sheep rumen, including a new species. J. Gen. Microbiol., 90. 100.

Kepler, C.R., Tove, S.B. (1967a). Biohydrogenation of unsaturated fatty acids. J. Biol. Chem., 241. 1351-1354.

Kepler, C.R., Tove, S.B. (1967b). Biohydrogenation of unsaturated fatty acids. J. Biol. Chem., 242. 5686.

Kepler, C.R., Tucker, W.P., Tove, S.B. (1971). Biohydrogenation of unsaturated fatty acids. J. Biol. Chem., 246. 2765-2771.

Lin, H., Boylston, T.D., Chang, M.J., Luedecke, L.O., Schultz, T.D. (1995). Survey of the conjugated linoleic acid contents of dairy products. J. Dairy Sci., 78. 2358-2365.

Palmquist, D.L., Schanbacher, F.L. (1991). Dietary fat composition influences fatty acid composition of milk fat globule membrane in lactating cows. Lipids, 26. 718.

Pariza, M.W., Hargraves, W.A. (1985). A beef-derived mutagenesis modulator inhibits initiation of mouse epidermal tumours by 7,12 dimethylbenz(a)anthracene. Carcinogenesis, 6. 591-593.

Parodi, P.W. (1994). Conjugated linoleic acid: An anticarcinogenetic fatty acid present in milk fat. Journal of Dairy Techology, 49. 93-97.

Polan, C.E., McNeill, J.J., Tove, S.B. (1964). Biohydrogenation of unsaturated fatty acids by rumen bacteria. J. Bacteriol., 88. 1056.

Pollard, M.R., Gunstone, F.D., James, A.T., Morris, L.J. (1980). Desaturation of positional and geometric isomers of monoenoic fatty acids by microsomal preparations from rat liver. Lipids, 15. 306-314.

Precht, D., Molkentin, J. (2000). Frequency distributions of conjugated linoleic acid and trans fatty acid contents in European bovine milk fats. Milchwissenschaft, 55. 12. 687-691.

Riel, R.R. (1963). Physico-chemical characteristics of Canadian milk fat. Unsaturated fatty acids. J. Dairy Sci., 46. 102-106.

Romero, K.P., Rizvi, S.S.H., Kelly, M.L., Bauman, D.E. (2000). Short communication: concentration of conjugated linoleic acid from milk fat with a continuous supercritical fluid processing system. J. Dairy Sci., 83. 20-22.

Shorland, F.B., Weenink, R.O., Johns, A.T. (1955). Effect of the rumen on the dietary fat. Nature, 175. 1129.

Schultz, T.D., Chew, B.P., Seaman, W.R., Luedecke, L.O. (1992). Inhibitory effect of conjugated dienoic derivatives of linoleic acid and ß-carotene on the in vitro growth of human cancer cells. Cancer Lett., 63. 125-133.

Wolff, R.L., Bayard, C.C., Fabien, R.J. (1995). J. Am. Oil. Chem. Soc., 72. 1471-1482.

acs68_51