CLA: What is it, and

Available evidence leaves little doubt that CLA can have a major effect on improving certain health problems in people. It has also been shown that CLA can have an impact on changing the saturation levels of body fat in pigs.

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OVER the past several years, much has been reported about a unique set of fatty acids classified as conjugated linoleic acids (CLA).

There are many published references about the benefits to both people and animals of incorporating CLA into their dietary regimes. There are also a large number of publications that describe the chemistry and functionality of CLA. The purpose of this article is to present a summary of the published information that describes and defines CLA, how it functions and its potential benefits when it is incorporated into the diets of people and animals.

Functional isomers

CLAs are a group of polyunsaturated fatty acids that are positional and geometric isomers of linoleic acid (C18:2). The Figure depicts the structures of linoleic acid and two different CLA isomers. The double bonds of linoleic acid are separated by a methylene group, whereas the double bonds in CLA are conjugated — that is, they are not separated by a methylene group and occur in a continuous configuration (Kritchevsky, 2000).

There are many different isomers of CLA found in nature. Of the natural sources, CLA is primarily found in ruminant meat and milk products (Wang and Jones, 2004; House et al., 2005).

As shown in the Table, 14 individual CLA isomers have been identified in milk products derived from dairy cows (Lock and Bauman, 2004).

CLA is naturally produced during bacterial fermentation in the rumen of ruminant animals. The biohydrogenation of linoleic acid from the diet is the first step in ruminant CLA synthesis. It produces cis-9, trans-11 CLA, also known as rumenic acid, and is the result of isomerization of the delta-12 double bond by linoleate isomerase (Bauman et al., 1999; Wahle et al., 2004). Rumenic acid is further hydrogenated to produce trans-11 18:1 vaccenic acid. Variability in these reactions results for the different isomers and concentrations of CLA in ruminant products (Wahle et al., 2004).

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Beneficial effects

The two primary CLA isomers thought to be the most biologically active are cis-9, trans-11(c9t11) and trans-10, cis-12 (t10c12; Figure). The main isomer produced by ruminants is rumenic acid (c9t11, about 90% of total CLA; Wahle et al., 2004).

Research has been conducted with laboratory animals, cultured cells and people on the effects of CLA and has shown beneficial effects of CLA against obesity, cancer, atherosclerosis and diabetes (Belury, 2002; Wang and Jones, 2004; House et al., 2005). The wide array of CLA effects are mainly attributed to the differences between isomers, and, thus, differences in findings are sometimes due to the amount and composition of CLA supplemented.

The c9t11 isomer has a unique ability to be readily incorporated into phospholipids of cell membranes, which has led researchers to postulate that this may contribute to the apparent biological effects of the reduction in atherosclerosis and some forms of cancer in laboratory animals and cell line models (Belury and Kempa-Steczko, 1997).

Conversely, the t10c12 isomer results in decreased fat accretion, perhaps due to increases in fat mobilization and oxidation, along with increases in energy expenditure (Wahle et al., 2004).

Many studies have shown that CLA mixtures are able to reduce adipose tissue depots in rodents, pigs and people and that this effect is specific to the t10c12 isomer or a mixture containing greater than 50% t10c12 (Belury, 2002; Wang and Jones, 2004). Postweanling mice fed 1% CLA for 28-30 days had a 50% reduction in total adipose tissue compared to control mice (Park et al., 2001).

In pigs, CLA inclusion in feed has resulted in decreased back fat thickness in grow-finish pigs (Tischendorf et al., 2002; Wiegand et al., 2002). Overweight or obese people given CLA for 12 weeks had reduced body fat mass, but their body mass index remained unchanged (Blankson et al., 2000).

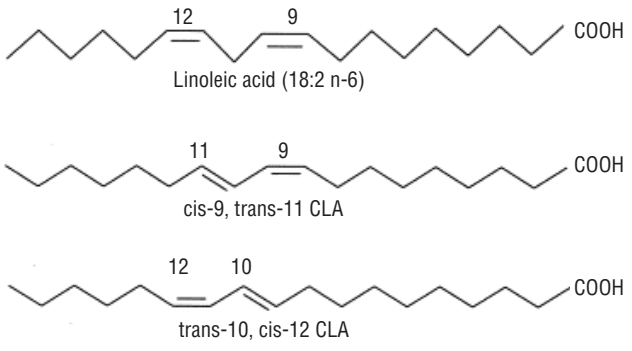
As previously mentioned, a noted effect of CLA is the inhibition of cancer, specifically mammary, prostate, skin, colon and stomach cancers (Belury, 2002). The anti-carcinogenic effects of CLA have been mainly attributed to the c9t11 isomer (Wang and Jones, 2004). In studies of mammary and prostate cancer cell model lines, feeding 1% CLA significantly reduced the growth of cancerous cells. Other studies of the same cell lines have not demonstrated these effects (Belury, 2002).

Several studies have also demonstrated tumor growth inhibitory effects and cell proliferation inhibitory effects of CLA in animal and cell models (Wahle et al., 2004). These effects have made CLA a candidate for nutritional intervention prior to or in conjunction with chemotherapeutic agents given to cancer patients, potentially reducing the amount of the standard drug used and, thus, reducing the associated side effects (Wahle et al., 2004).

CLA reduces atherosclerotic plaque formation (Belury, 2002). Inclusion of 0.5 g per day in hypercholesterolemic diets fed to rabbits for 12 weeks resulted in significantly reduced serum triacylglycerols, low-density lipoprotein (LDL) cholesterol levels and atherosclerotic plaque formation in the aorta (Lee et al., 1994). Other studies have shown reduced plaque formation, inhibited cytokine formation and inhibited angiogensis (Wahle et al., 2004). The reduction of plaque deposits by CLA was proposed to be due to changes in LDL oxidative susceptibility (Belury, 2002).

Effects of CLA on the onset of diabetes and insulin resistance are inconsistent. Rats fed CLA have shown significantly reduced fasting glucose, insulinemia, triglyceridemia, free fatty acids and leptinemia

1. Structure of linoleic acid, cis-9, trans-11 CLA and trans-10, cis-12 CLA (Evans et al., 2002)



Range of CLA isomers found in conjugated 18:2 fatty acid in milk

Isomer	% of total CLA isomers*
Trans-7, cis-9	1.20-8.89
Trans-7, trans-9	0.02-2.39
Trans-8, cis-10	0.06-1.47
Trans-8, trans-10	0.19-0.37
Cis-9, trans-11	72.56-91.16
Trans-9, trans-11	0.77-2.87
Trans-10, cis-12	0.03-1.51
Trans-10, trans-12	0.28-1.31
Cis-11, trans-13	0.18-4.70
Trans-11, cis-13	0.07-8.00
Trans-11, trans-13	0.28-4.24
Cis-12, trans-14	0.04-0.80
Trans-12, trans-14	0.33-2.76
Cis, cis isomers	0.0-4.80

*Data derived from studies in which FA analyses were carried out on milk, cheese and butter samples.
Table adapted from Lock and Bauman, 2004.

Live-animal test to detect scrapie developed

GOATS are tough, spirited animals, but they're no match for scrapie, a form of transmissible spongiform encephalopathy.

Now, Agricultural Research Service (ARS) scientists and collaborators have developed a live-animal test to detect scrapie in goats.

Called the rectal mucosa biopsy test, or rectal biopsy, the new method involves snipping a tiny piece of lymphoid tissue from the lining of a suspected animal's rectum. A dab of local anesthetic eases the animal's discomfort, noted microbiologist Katherine O'Rourke with the ARS Animal Diseases Research Unit in Pullman, Wash.

Lymphoid tissue is used because it collects malformed proteins called prions, which are thought to cause scrapie, O'Rourke added.

O'Rourke is a member of a scrapie research team that includes Washington State University, Colorado State University, the Animal & Plant Health Inspection Service (APHIS), the National Park Service and the Canadian Food Inspection Agency.

Advantages of using the rectal biopsy test method include its speed, easier methodology and its generation of a high number of repeat samples from individual animals.

On a related front, ARS Pullman geneticist Stephen White is leading studies to characterize the prion protein gene of goats and identify differences between individual animals and breeds harboring the gene.

His team has so far examined the sequences and distribution of alleles — alternative forms of genes — from 446 goats representing 10 breeds, including Alpine, Angora, Boer and Nubian.

The ARS Pullman lab also is collaborating with APHIS to formulate a strategy aimed at helping the goat industry eliminate scrapie from the U.S. herd, which numbers 4 million head.

Hardships imposed by scrapie on America's goat and sheep producers include the physical loss of animals, costs of carcass and offal disposal, trade restrictions and diminished domestic and international markets for breeding stock, semen and embryos. ■

how does it work?

(Belury, 2002). Butter enriched with c9t11 CLA failed to reduce glucose tolerance, lower adipose tissue or enhance glucose uptake, leading to the conclusion that perhaps it is the t10c12 isomer that is responsible for the antidiabetogenic responses (Belury, 2002).

Insulin tolerance testing on CLA-fed mice showed marked insulin resistance without changes to blood glucose concentrations after oral glucose tolerance testing (Tsuboyama-Kasaoka et al., 2000). Other studies have examined the reduction of plasma leptin by CLA and the concomitant changes in blood glucose levels due to regulation by leptin (Wang and Jones, 2004). Feeding male mice high-fat diets with 1% CLA resulted in reduced plasma leptin levels in one study (DeLany et al., 1999) while resulting in no change in plasma leptin or glucose levels in another (West et al., 2000).

Mechanism of CLA

The primary mechanism of the metabolic regulation of CLA is believed to be a group of nuclear transcription factors, i.e., peroxisome proliferator-activated receptors (PPARs; Belury, 2002). Several genes involved in the transcription of beta-oxidation enzymes contain a functional PPAR element in their enhancing regions, i.e., acyl-CoA oxidase, liver fatty acid-binding protein, lipoprotein lipase and others (Schoonjans et al., 1996).

CLA or its metabolites are high-affinity ligands and activators of PPAR-alpha that induce accumulation of PPAR-responsive mRNAs in hepatic cells (Moya-Camarena et al., 1999). However, recent studies have shown that mice lacking the PPAR-alpha expression but expressing PPAR-beta/PPAR-delta and PPAR-gamma had similar responses such as a reduction in adipose tissue and an induction of some PPAR-responsive genes in the liver (Peters et al., 2001).

It is possible that CLA affects lipid metabolism via other isoforms of PPAR-alpha such as those mentioned above. For example, the cis-10 trans-12 isomer of CLA decreases the expression of PPAR-gamma in adipose tissue and increases the expression of PPAR-alpha in liver tissue (Moya-Camarena et al., 1999; Evans et al., 2002; Kang et al., 2003). By acting as a PPAR-gamma modulator, CLA is able to prevent lipid accumulation, as shown in cultured adipocytes (Granlund et al., 2003).

In addition to regulating PPARs, CLA is believed to inhibit stearoyl-CoA desaturase (SCD) activity. SCD is a key enzyme involved in the synthesis of monounsaturated fatty acids and their regulation. Alterations in this enzyme affect the saturated: unsaturated fatty acid ratio (Lee et al., 1998). Increasing the ratio of saturated to unsaturated fatty acids in the fat has been shown to increase fat firmness — as quantified by iodine value and the delta-9 desaturase index — in meat animals (Gatlin et al., 2002; Weber et al., 2006).

Previous studies have indicated that CLA tends to decrease both SCD-1 (Smith et al., 2002) and the delta-9 desaturase index in pigs (Demaree et al., 2002; Smith et al., 2002). Reductions in SCD-1 expression were seen with CLA feeding in both a mouse's liver and cultured preadipocytes (Belury, 2002). Decreasing SCD-1 mRNA expression, and thereby decreasing the amount of saturated fatty acids being converted to unsaturated fatty acids, may be responsible for the increased levels of saturated fatty acids seen after feeding CLA (Demaree et al., 2002; Smith et al., 2002).

Subcutaneous fat of pigs fed CLA had increased C14:0, C18:1 and C18:2 fatty acids. The increased C14:0 is most likely due to CLA inhibiting the desaturase enzyme, thereby decreasing the amount of monounsaturated fatty acids derived from C14:0 (Thiel-Cooper et al., 2001).

Additionally, feeding dietary CLA in several species alters the activity of fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC) in adipose and liver. CLA decreases mRNA for FAS and ACC to significantly inhibit the capacity for *de novo* lipogenesis (Ostrowska et al., 1999; House et al., 2005).

In barrows and gilts fed 0.25% or 0.50% CLA for the finishing diet from 97 kg to 172 kg, ACC activity was significantly reduced compared to control pigs (Corino et al., 2003).

Summary

CLA occurs naturally and is found in milk and beef products from cattle fed conventional diets of forage and grain supplements. There is a growing body of evidence that describes the potential benefits for the use of CLA as part of a dietary regimen for both people and animals.

The available evidence leaves little doubt that CLA can have a major positive effect on improving certain health problems in people. Research in laboratory animals has shown that CLA can play a role in reducing the effects of certain cancers, atherosclerosis, obesity and diabetes.

It has also been shown that CLA can have an impact on changing the saturation levels of body fat in pigs. CLA was recently approved by the Food & Drug Administration for use in growing and finishing swine diets.

References

Bauman, D.E., L.H. Baumgard, B.A. Corl and J.M. Griinari. 1999. Biosynthesis of conjugated linoleic acid in ruminants. Proceedings of the American Society of Animal Science.

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BELURY, M.A. 2002. Dietary conjugated linoleic acid in health: Physiological effects and mechanisms of action. Annu. Rev. Nutr. 22:505-531.

Belury, M.A., and A. Kempa-Steczko. 1997. Conjugated linoleic acid modulates hepatic lipid composition in mice. Lipids. 32:199-204.

Blankson, H., J.A. Stakkestad, H. Fagertun, E. Thom, J. Wadstein and O. Gudmundsen. 2000. Conjugated linoleic acid reduces body fat mass in overweight and obese humans. J. Nutr. 130:2943-2948.

Corino, C., S. Magni, G. Pastorelli, R. Rossi and J. Mourot. 2003. Effect of conjugated linoleic acid on meat quality, lipid metabolism and sensory characteristics of dry-cured hams from heavy pigs. J. Anim. Sci. 81:2219-2229.

Cox, A.D. 2005. Added dietary fat effects on market pigs and sows. M.S. thesis, Purdue University, West Lafayette, Ind.

DeLany, J.P., F. Blohm, A.A. Truett, J.A. Scimeca and D.B. West. 1999. Conjugated linoleic acid rapidly reduces body fat content in mice without affecting energy intake. Am. J. Physiol. 276:R1172-R1179.

Demaree, S.R., C.D. Gilbert, H.J. Mersmann and S.B. Smith. 2002. Conjugated linoleic acid differentially modifies fatty acid composition in subcellular fractions of muscle and adipose tissue but not adiposity of postweanling pigs. J. Nutr. 132:3272-3279.

Dugan, M.E.R., J.L. Aalhus and B. Uttaro. 2004. Nutritional manipulation of pork quality: Current opportunities. Advances in Pork Production. 15:237.

Eggert, J.M., M.A. Belury, A. Kempa-Steczko, S.E. Mills and A.P. Schinckel. 2001. Effects of conjugated linoleic acid on the belly firmness and fatty acid composition of genetically lean pigs. J. Anim. Sci. 79:2866-2872.

Evans, M.E., J.M. Brown and M.K. McIntosh. 2002. Isomer-specific effects of conjugated linoleic acid (CLA) on adiposity and lipid metabolism. J. Nutr. Biochem. 13:508-516.

Granlund, L., L.K. Juvet, J.I. Pedersen and H.I. Nebb. 2003. Trans-10, cis-12 conjugated linoleic acid prevents triacylglycerol accumulation in adipocytes by acting as a PPAR-gamma modulator. J. Lipid Res. 44:1441-1452.

House, R.L., J.P. Cassady, E.J. Eisen, M.K. McIntosh and J. Odle. 2005. Conjugated linoleic acid evokes de-lipidation through the regulation of genes controlling lipid metabolism in adipose and liver tissue. Obes. Rev. 6:247-258.

Kang, K., W. Liu, K.J. Albright, Y. Park and M.W. Pariza. 2003. Trans-10, cis-12 CLA inhibits differentiation of 3T3-L1 adipocytes and decreases PPAR-gamma expression. Biochem. Biophys. Res. Commun. 303:795-799.

Kritchevsky, D. 2000. Antimutagenic and some other effects of conjugated linoleic acid. Br. J. Nutr. 83:459-465.

Lee, K.N, D. Kritchevsky and M.W. Pariza. 1994. Conjugated linoleic acid and atherosclerosis in rabbits. Atherosclerosis. 108:19-25.

Lock, A.L., and D.E. Bauman. 2004. Modifying milk fat composition of dairy cows to enhance fatty acids beneficial to human health. Lipids. 39:1197-206.

Moya-Camarena, S.Y., J.P. Vanden Heuvel, S.G. Blanchard, L.A. Leesnitzer and M.A. Belury. 1999. Conjugated linoleic acid is a potent naturally occurring ligand and activator of PPAR-alpha. J. Lipid Res. 40:1426-1433.

Ostrowska, E., M. Muralitharan, R.F. Cross, D.E. Bauman and F.R. Dunshea. 1999. Dietary conjugated linoleic acids increase lean tissue and decrease fat deposition in growing pigs. J. Nutr. 129:2037-2042.

Park, Y., K.J. Albright, J.M. Storkson, W. Liu, M.E. Cook and M.W. Pariza. 2001. Changes in body composition in mice during feeding and withdrawal of conjugated linoleic acid. Lipids. 34:243-248.

Schoojans, K., B. Staels and J. Auwerx. 1996. The peroxisome proliferators activated receptors (PPARS) and their effects on lipid metabolism and adipocyte differentiation. Biochimica et Biophysica Acta. 1302:93-109.

Smith, S.B., T.S. Hively, G.M. Cortese, J.J. Han, K.Y. Chung, P. Castenada, C.D. Gilbert, V.L. Adams and H.J. Mersmann. 2002. Conjugated linoleic acid depresses the delta9 desaturase index and stearoyl coenzyme A desaturase enzyme activity in porcine subcutaneous adipose tissue. J. Anim. Sci. 80:2110-2115.

Thiel-Cooper, R.L., F.C. Parrish Jr., J.C. Sparks, B.R. Wiegand and R.C. Ewan. 2001. Conjugated linoleic acid changes swine performance and carcass composition. J. Anim. Sci. 79:1821-8.

Tischendorf, F., F. Schone, U. Kirchheim and G. Jahreis. 2002. Influence of a conjugated linoleic acid mixture on growth, organ weights, carcass traits and meat quality in growing pigs. J. Anim. Physiol. Anim. Nutr. (Berl.). 86:117-128.

Tsuboyama-Kasaoka, N., M. Takahashi, K. Tanemura, H.J. Kim, T. Tange, H. Okuyama, M. Kasai, S. Ikemoto and O. Ezaki. 2000. Conjugated linoleic acid supplementation reduces adipose tissue by apoptosis and evelops lipodystrophy in mice. Diabetes. 49:1534-1542.

Wahle, K.W., S.D. Heyes and D. Rotondo. 2004. Conjugated linoleic acids: Are they beneficial or detrimental to health? Prog. Lipid Res. 43:553-587.

Wang, Y.W., and P.J.H. Jones. 2004. Conjugated linoleic acid and obesity control: Efficacy and mechanisms. Int. J. Obes. 28:941-955.

West, D.B., F.Y. Blohm, A.A. Truett and J.P. DeLany. 2000. Conjugated linoleic acid persistently increases total energy expenditure in AKR/J mice without increasing uncoupling protein gene expression. J. Nutr. 130:2471-2477.

Wiegand, B.R., J.C. Sparks, F.C. Parrish Jr. and D.R. Zimmerman. 2002. Duration of feeding conjugated linoleic acid influences growth performance, carcass traits and meat quality of finishing barrows. J. Anim. Sci. 80:637-643. ■