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Conjugated linoleic acid

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Introduction

Conjugated linoleic acid (CLA), first positively identified in 1987 (Ha *et al.*), is a collective term describing the positional and geometric conjugated dienoic isomers of linoleic acid. Linoleic acid (C18:2) has double bonds located on carbons 9 and 12, both in the *cis* (*c*) configuration, whereas CLA has either the *cis* or *trans* (*t*) configuration or both located along the carbon chain. Sources of CLA have been shown to elicit many favorable biological responses including: (i) increased rate and (or) efficiency of gain in growing rats (Chin *et al.*, 1994) and pigs (Dugan *et al.*, 1997; Thiel *et al.*, 1998; O'Quinn PR, Waylan AT, Nelssen JL *et al.*, submitted for publication); (ii) reduced fat deposition and increased lean in mice (Park *et al.*, 1997) and pigs (Dugan *et al.*, 1997; Thiel *et al.*, 1998; O'Quinn *et al.*, 2000a); (iii) improved immune function in rats and chicks (Cook *et al.*, 1993; Sugano *et al.*, 1998); and (iv) reduced atherosclerosis in rabbits (Lee *et al.*, 1994) and hamsters (Nicolosi *et al.*, 1997). Conjugated linoleic acid is also a potent anticarcinogen *in vivo* and *in vitro* (Ha *et al.*, 1990; Ip *et al.*, 1991; Durgam and Fernandes, 1997) and may exhibit some antioxidant properties (Decker, 1995), possibly as a result of its involvement in the metabolism of α-tocopherol (O'Quinn *et al.*, 1999). Additionally, CLA increases adipocyte insulin sensitivity (Houseknecht *et al.*, 1998b) and, therefore, has become a highly studied factor for the management of type I (Collier *et al.*, 1988) and type II (Hendra *et al.*, 1991; Singh *et al.*, 1992) diabetes mellitus, a disease affecting over 100 million people in the United States alone (Pickup and Crook (1998).

Discovery and background

While investigating the potential formation of mutagens in meat during cooking, researchers at the University of Wisconsin, Madison, discovered a mutagen inhibitor, which they later positively identified as CLA (Ha *et al.*, 1987). Naturally occurring CLA has since been identified in many different meat and dairy products (Chin *et al.*, 1991; Parodi, 1994; Lin *et al.*, 1995). Conjugated linoleic acid is a non-specific term that refers to any of the positional and geometric isomers of α-linoleic acid (*c*9,*c*12-octadecadienoic acid). The double bonds in CLA occur predominantly at carbon positions 9 and 11 or 10 and 12. Thus, the original nomenclature for CLA gave rise to eight theoretically possible isomers (*c*9,*c*11; *c*9,*t*11; *t*9,*c*11; *t*9,*t*11; *c*10,*c*12; *c*10,*t*12; *t*10,*c*12; and *t*10,*t*12) of linoleic acid (Ip *et al.*, 1991). However, as the depth of research and analytical capabilities have increased, more CLA isomers have been identified (Sehat *et al.*, 1998; Yurawecz *et al.*, 1998). Figure 1 shows a diagram of linoleic acid and the *c*9,*t*11 configuration of CLA.

Sources of CLA

CLA is naturally present in many types of meats and dairy products and is also manufactured for use in dietary supplements for experimental use by several methods and from many substrates.

Dietary CLA

Since the discovery of CLA in fried ground beef (Ha *et al.*, 1997), much research has focused on determining the CLA content of foods. Ruminant tissues contain more CLA than non-ruminant tissues, which have average values of 4.5 and less than 1 mg CLA/g of fat respectively (Ha *et al.*, 1989). By contrast, dairy products are the richest natural source of CLA, but the content varies widely depending upon pasture conditions (Parodi, 1994) and ranges from 4.2 mg/g of lipid in fermented dairy products (Lin *et al.*, 1995) to 30 mg/g of lipid in milk fat (Parodi, 1994). Conjugated linoleic acid may also be present in some vegetable products and infant foods at low concentrations (Fogerty *et al.*, 1988). However, meat and dairy products represent the largest contributors of naturally occurring CLA. Non-ruminants incorporate CLA into their tissues

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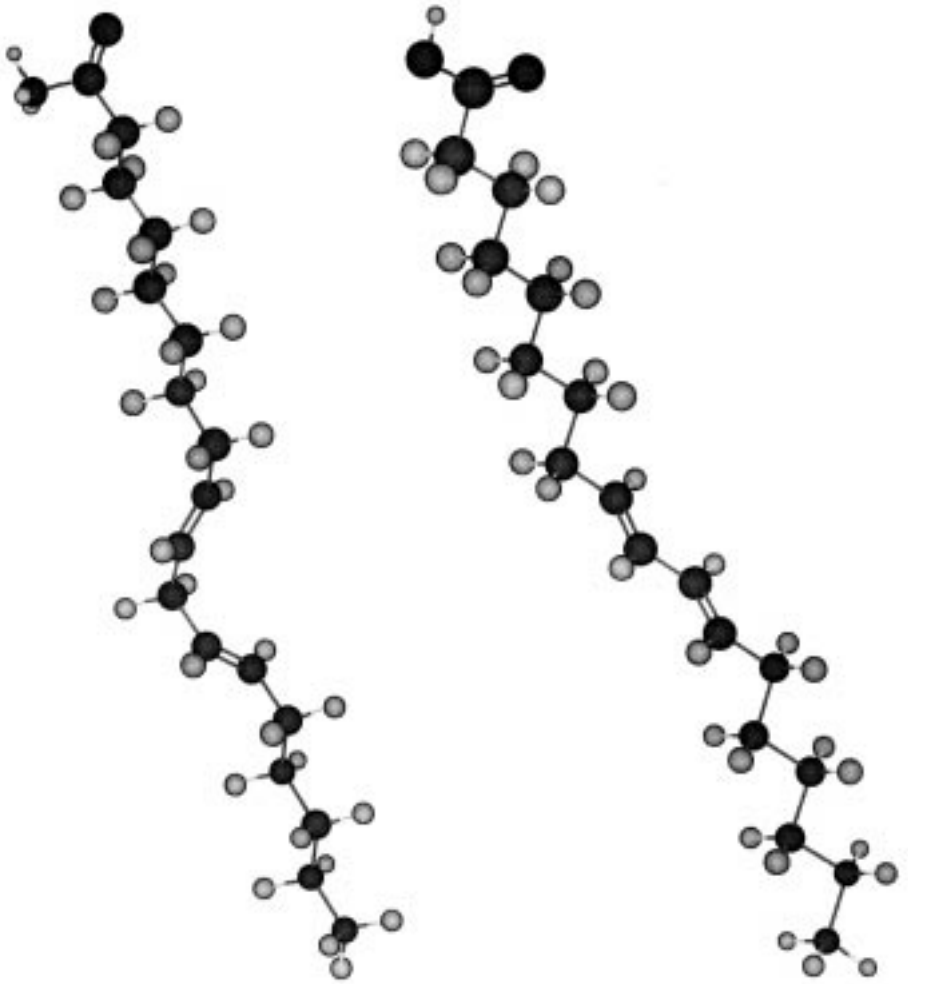


Fig. 1. Diagram of linoleic and conjugated linoleic acids.

through the consumption of dietary CLA and from small amounts of bacterial isomerization of linoleic acid. In contrast, ruminants incorporate CLA primarily by the biohydrogenation of linoleic acid and to a lesser extent via dietary intake. The *c*9,*t*11 form of CLA is produced as a first intermediate in the biohydrogenation of dietary linoleic acid by a linoleic isomerase produced in the rumen by *Butyrivibrio fibrisolvens* (Parodi, 1994). Thus, *c*9,*t*11 is the predominant isomer in the milk of ruminants. Consumption of dairy products with a high CLA content (e.g. cheddar cheese) has been shown to elevate plasma CLA concentrations effectively in healthy men (Huang *et al*., 19994). Thus, dietary modification may be an efficient (though not widely accepted) method of increasing CLA levels in humans (McGuire and McGuire, 1999).

Manufactured CLA

The majority of work conducted with CLA before 1997 involved its synthesis from linoleic acid, which has been described in detail (Ip *et al*., 1991). Briefly, 500 g linoleic acid (at least 99% pure) is added to a 5-litre, three-neck flask containing 150 g sodium hydroxide dissolved in 2900 g ethylene glycol. The mixture is heated at 180°C under an inert atmosphere for 2 hours. The reaction mixture is cooled to ambient temperature, and 320 ml of concentrated hydrochloric acid is added. After 15 minutes of stirring, the pH of the mixture is adjusted to 4 with hydrochloric acid. The reaction mixture is then transferred to a 4-litre separatory funnel and extracted with two 500-ml portions of hexane. The new solution is then extracted with three 250-ml portions of 5% sodium chloride, dried over 3-Å molecular sieves, filtered through a sintered glass funnel, and placed in a rotary evaporator. After the removal of the hexane, the resulting CLA mixture is ready for dietary incorporation and other research needs, such as infusion or use in a bolus. This method has been reported widely (Ha *et al*., 1990; Ip *et al*., 1991, 1994a; Chin *et al*., 1994). Obviously, this tedious process precludes the feeding of CLA to livestock, because of the limited quantities that can be

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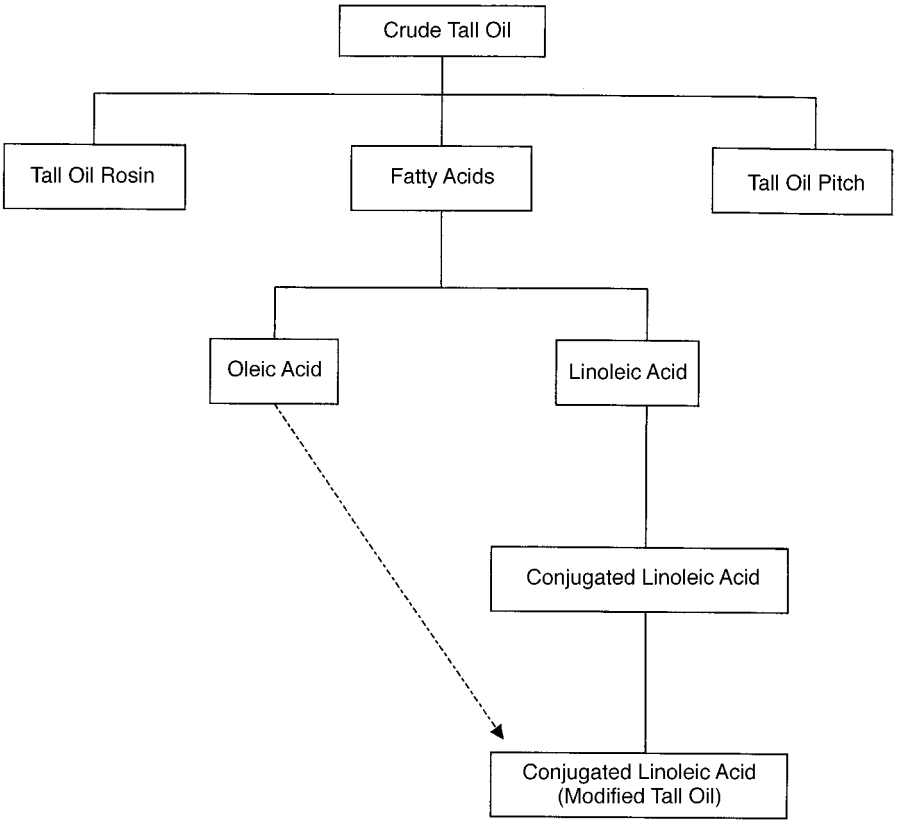


Fig. 2. Production of modified tall oil from crude tall oil.

produced. In the mid-1990s, a European company started producing large quantities of CLA from sunflower oil (a rich source of α-linoleic acid). This CLA was subsequently fed to large numbers of pigs, and results were reported in 1997 (Dugan *et al.*, 1997). In 1998 in the USA, CLA derived from sunflower oil was also fed to pigs (Thiel *et al.*, 1998; O'Quinn *et al.*, 2000a). Now, most large, diversified, chemical suppliers manufacture CLA. For large-scale studies requiring large amounts of CLA, the precursor has to be sunflower oil or tall oil (a by-product of kraft pulping of pine wood (Huibers, 1997). Modified tall oil (MTO) has been shown to be an effective dietary source of CLA for rats (O'Quinn *et al.*, 1999) and pigs (Waylan, 1999; Woodworth *et al.*, 1999; O'Quinn *et al.*, 1999b, 2000a–c). Figure 2 illustrates the production of MTO from crude tall oil. Pigs fed MTO have better growth performance than pigs fed CLA derived from sunflower oil (O'Quinn *et al.*, 2000a). Worldwide production of crude tall oil in 1995 was 1.7 million metric tons. Tall oil is surprisingly heat-stable and does not degrade when fractionally distilled into tall oil fatty acids (about 49% oleic and 45% linoleic acids) or tall oil resins/rosins (Huibers, 1997). Because tall oil is a by-product of the pulp and paper industry, it could provide an abundant and economic alternative to CLA derived from sunflower oil in feeding studies conducted with livestock species. MTO would need to be purified further before it could be used in human applications. Commercial production of MTO or CLA from sunflower oil yields a product that contains appreciable amounts of neutrals or unsaponifiable matter (1–6%). This variability in the raw material led to the initial rejection of tall oil fatty acids as feed additives in 1985 (AAFCO, 1985), although the composition of the neutrals in pine tall oil is well characterized (Conner and Rowe, 1975) and tall oil phytosterols have been used recently to lower cholesterol levels in human patients (Jones *et al.*, 1998). Clearly, the unsaponifiable matter in MTO and CLA from sunflower oil will have to be identified and characterized before the approval of either for use as a feed additive for any livestock species.

Methods of CLA analysis

Though analytical capabilities continue to improve, a consensus on CLA analyses has not been achieved yet. CLA analysis in early studies used either gas chromatography (GC) (Ha *et al.*, 1990; Ip *et al.*, 1991, 1994a) or high-performance liquid chromatography (HPLC) (Ha *et al.*, 1990). Official analytical handbooks (AOCS, 1994; AOAC, 1995) recommend the quantitation of CLA isomers by GC analysis of their fatty acid methyl esters (FAME) using long (i.e. 100-meter), polar capillary

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columns. The observed GC retention times are compared against those of commercially available CLA standards. The various methods employed to make the FAME have also been scrutinized (Shantha *et al.*, 1993; Kramer *et al.*, 1997). Production of the FAME may require acid- and(or) base-catalysed methylation. The AOAC (AOAC, 1995) and AOCS (AOCS, 1994) methods for FAME use alcoholic sodium hydroxide for transesterification of esterified fat followed by treatment with BF_3-MeOH. Typical fatty acids are not affected by this process. However, the conjugated double bonds of CLA are labile and can be destroyed by treatment with BF_3-MeOH, and increased amounts of *trans,trans* double bonds can be produced erroneously. The AOAC (AOAC, 1995) and AOCS (AOCS, 1994) acknowledge this limitation, although numerous research groups still employ this procedure for CLA analysis (Ha *et al.*, 1987, 1989, 1990; Ip *et al.*, 1991; O'Quinn *et al.*, 2000a). This method of analysis is still useful as long as the results are used only for internal comparisons and not for pinpoint comparisons against external research. Recently, a silver-ion high-performance liquid chromatography technique (Ag^+-HPLC) was developed (Sehat *et al.*, 1998) that resolves CLA isomers on the basis of chain length, double-bond configuration and the position of the conjugated diene functional group in the fatty acid chain. This method was reported to yield substantially better results than GC or HPLC with C_{18} silica columns, mentioned above. Research from our laboratory (O'Quinn *et al.*, 2000a) and others (Kramer *et al.*, 1998; Yurawecz *et al.*, 1998) has indicated that a combination of GC (using 100-m capillary columns) and Ag^+-HPLC is necessary to obtain a complete profile of a sample of CLA or MTO.

Specific isomers of CLA

In addition to the identification of additional isomers of CLA (Sehat *et al.*, 1998; Yurawecz *et al.*, 1998), research has also focused on verifying the functionality and activity of these isomers. As previously mentioned (IP *et al.*, 1991), the earlier work was based on the eight theoretically possible isomers of linoleic acid. When CLA was synthesized from linoleic acid, 90% of the composition consisted of *c*9,*t*11; *t*10,*c*12; *t*9,*t*11; and *t*10,*t*12 isomers, and the remaining 10% of *c*9,*c*11; *t*9,*c*11; *c*10,*c*12; and *c*10,*t*12 isomers[27]; the *c*11,*c*13 isomer was also found to be a component of this minor fraction (Ha *et al.*, 1990). Several studies were conducted using specifically prepared CLA that consisted of the four predominant isomers (Ha *et al.*, 1987; Sugano *et al.*, 1997). Interestingly, the isomeric profiles of CLA derived from sunflower oil and MTO used in prior work with pigs (O'Quinn *et al.*, 2000a) do not follow this same pattern. For both sources, the *c*10,*c*12 isomer was present at about 20% of the total, whereas the *t*10,*t*12 isomer fell within the minor fraction, which included other isomers at about 10% of the total. The total concentration of CLA can be controlled in the process of manufacturing CLA from sunflower oil or MTO from tall oil, but the amount of isomerization cannot. Thus, variations in isomeric profiles may be expected. However, the MTO used in studies with rats (O'Quinn *et al.*, 1999a) and pigs (O'Quinn *et al.*, 1999b, 2000b, c) had very similar isomeric profiles. As recently as 1998, no direct evidence existed about the biologically active isomer(s) of CLA (Sehat *et al.*, 1998), but the *c*9,*t*11 isomer was assumed to be biologically active, on the basis solely of its predominance in milk and dairy products. Recent work in mice (Park *et al.*, 1999a, b) has focused on feeding predominantly the *c*9,*t*11 and *t*10,*c*12 isomers, and has concluded that the *t*10,*c*12 isomer produces the changes in body composition that are observed routinely in mice, rats and pigs. Additionally, studies using Holstein cows have indicated that the *t*10,*c*12 isomer is responsible for the antiobesity effects of CLA in growing animals (Baumgard *et al.*, 1999). A recent study also concluded that the *c*9,*t*11 isomer was responsible for the anticarcinogenic effects of CLA in animals (Bauman and Griinari, 1999). However, these results do not seem to apply when commercially available CLA derived from sunflower oil or MTO is fed to pigs (O'Quinn *et al.*, 2000a). Pigs fed diets containing MTO or CLA that had similar concentrations of *c*9,*t*11 (20.52 and 21.33% respectively) and *t*10,*c*12 (14.37 and 16.40% respectively) had significantly different growth performance, pigs fed diets containing MTO having significantly higher average daily gain (ADG) and average daily feed intake (ADFI), but no differences in body composition. However, the contents of other isomers differed substantially between MTO and CLA (e.g. 14.80 and 3.90% *t*9,*t*11 respectively). These data suggest that CLA or MTO produced in bulk quantities on a large scale differ in the biological responses they elicit and do not support the use of *c*9,*t*11 or *t*10,*c*12 alone or in conjunction as the biologically active isomers. Until the active isomer(s) are known definitively, the best approach is to feed a source of CLA or MTO that contains many isomers in addition to those thought to be biologically active. However, when CLA is produced under closely controlled conditions, the *c*9,*t*11 and *t*10,*c*12 isomers are apparently effective in controlling carcinogenesis and adiposity. Thus, the optimum source of CLA may be dictated by the desired response in the recipient.

Conjugated linoleic acid and immune modulation

Estimates of yearly economic losses borne by animal producers in the United States as a result of immune stimulation have reached $500 million (Cook and Pariza, 1998). During typical immune stimulation, cells of the immune system interact with antigens and release cytokine signals to direct the immune response.

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Interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α) are two cytokines involved in the catabolism that is normally seen during immune responses. These cytokines are critical in the defense process, yet they redirect nutrient flow to immune-related products and induce the degradation of skeletal muscle and decreased muscle synthesis (Cook and Pariza, 1998). Thus, any immune stimulation (e.g. from the environment, vaccines or disease challenge) will impair the growth performance of livestock species. Cytokine-induced muscle degradation is associated with a rise in prostaglandin E_2 (PGE_2) levels. Eicosanoids and leukotrienes are metabolites of arachidonic acid, which is cleaved from phospholipids by phospholipase and, in the presence of cyclooxygenase, can be converted to PGE_2. Arachidonic acid is an elongated and desaturated product of linoleic acid, and therefore provides a plausible link for the effects of CLA on the immune system (Cook and Pariza, 1998). Macrophages produce PGE_2, IL-1 and TNF-α; PGE_2 downregulates the immune response and the release of cytokines. On the basis of this biochemical linkage, a series of experiments was conducted at the University of Wisconsin, Madison, to evaluate the ability of CLA to modulate the response of the immune system (Cook and Pariza, 1998). Mice fed CLA and injected daily with TNF-α lost less weight than their contemporaries that were not fed CLA. Similarly, in poultry, supplementation with CLA prevented the catabolic effects of immune stimulation [lipopolysaccharide (LPS) challenge] (Cook *et al*., 1993). However, the effects of CLA on immune function are not due to immune suppression. Studies have shown that CLA does not affect antibody responses to sheep red blood cells in chicks (Cook *et al*., 1993), and increases serum α-1-acylglycoprotein in pigs exposed to a dirty environment (Bassaganya *et al*., 1999). Additionally, CLA, alone or in conjunction with β-carotene, was reported to enhance several measures of immune responsiveness, including the killing ability of macrophages (Chew *et al*., 1997). Thus, somewhat paradoxically, CLA prevents immune-induced weight loss without compromising immune function. One study postulated that CLA accomplished this by affecting eicosanoid production as previously described and preventing the downregulation of the immune response and also muscle wasting (Cook and Pariza, 1998). Another study reported that CLA reduced the basal level of TNF-α but not the LPS-induced level of TNF-α (Turek *et al*., 1998). In the same study, CLA reduced both the basal and the LPS-induced level of IL-6, but had no effect on the IL-1 level and only marginally reduced the PGE_2 level. The authors concluded that the effects of CLA on PGE_2 production were diet- and tissue-dependent. The effects of CLA on the immune system may also be age-dependent (Hayek *et al*., 1999). Conjugated linoleic acid enhanced T-cell function to a greater extent in young than in old mice, but the changes were not mediated through alterations in IL-1 or PGE_2 production. CLA has also been shown to reduce the release of leukotriene B_4 and PGE_2 without affecting the release of histamine (Sugano *et al*., 1998). However, dietary supplementation with CLA increased the levels of immunoglobulins A, G and M but reduced the level of immunoglobulin E. It is apparent that CLA offers many benefits to animal producers through the modulation of the immune system, though the exact mechanisms behind its ability to counteract immune activation while improving immune responsiveness are still not understood clearly.

Conjugated linoleic acid and cancer

Since its discovery as a mutagen inhibitor (Ha *et al*., 1987), CLA has been studied extensively for its involvement in the modulation of cancer. Suppression of tumor growth by CLA has been noted for mammary cancer (Ip *et al*., 1991; Durgam and Fernandes, 1997; Cunningham *et al*., 1997; Ip and Scimeca, 1997), stomach cancer (Ha *et al*., 1990), skin cancer (Belury *et al*., 1996) and prostate cancer (Cesano *et al*., 1998). Because CLA affects a wide array of cancer cell lines, its mode of action could be non-specific or it could simply displace linoleic acid, which is the only essential fatty acid that increases mammary carcinogenesis (Ip *et al*., 1985). However, studies have shown that CLA modulates mammary carcinogenesis independently of dietary fat and does not displace linoleic or arachidonic acid (Ip and Scimeca, 1997). Although CLA seems to protect against extrahepatic cancer cell lines, it may increase the risk of hepatic cancer. CLA has been linked to increased peroxisome proliferation (Belury *et al*., 1997; Houseknecht *et al*., 1998b). This is a pleiotropic cellular response to a wide range of chemical compounds. Peroxisomal enzymes are involved in many catabolic and anabolic pathways, such as the β-oxidation of long-chain fatty acids, fatty acid elongation, acyl-CoA hydrolysis, the conversion of acyl-CoA to acylcarnitine, cholesterol biosynthesis, the catabolism of polyamines and amino acids, and the metabolism of reactive oxygen species. Peroxisome proliferation is limited to certain tissues, such as the liver and kidneys, and is somewhat species-specific, rats (particularly males) being the most susceptible (Schoonjans *et al*., 1996). Sustained peroxisome proliferation has been implicated in hepatocarcinogenesis (Kraupp *et al*., 1990), although peroxisome proliferators do not show detectable mutagenic or genotoxic activity in a suitable test environment. Conjugated linoleic acid is known to induce molecular markers of peroxisome proliferation [acyl-CoA oxidase (the peroxisome-specific enzyme), liver fatty acid binding protein (the lipid transporter) and cytochrome P4504A1 (the microsome-associated cytochrome)] and ornithine decarboxylase activity (associated with liver tumor promotion) by as much as tenfold (Belury *et al*., 1997). However, peroxisome proliferators are generally classified as non-genotoxic carcinogens; thus, they do not bind

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directly to DNA to initiate carcinogenesis. Instead, they promote tumorigenesis in the liver, testes and pancreas by promoting cell proliferation, altered cell differentiation and the inhibition of apoptosis of initiated cells (Reddy, 1990; Belury *et al.*, 1997). Prior work has shown that CLA can alter the proliferation and differentiation of adipocytes (Satory and Smith, 1999) and the distribution of cell size (Sisk *et al.*, 1996). However, not all hepatocarcinogens are peroxisome proliferators, and some peroxisome proliferators (e.g. linoleic and arachidonic acids) have little or no effect on liver tumorigenesis. Thus, the exact role of CLA in promoting liver carcinogenesis is still unclear (Belury *et al.*, 1997). Early work with CLA and cancer modulation was based on its proposed antioxidant properties. Initially (Ha *et al.*, 1990; Ip *et al.*, 1992; Decker, 1995), CLA was thought to be a potent antioxidant, but controlled experiments using physiological levels of CLA (van den Berg *et al.*, 1995) could not demonstrate an antioxidant property. However, in live animal models, CLA (Nicolosi *et al.*, 1997) and MTO (O'Quinn *et al.*, 1999; Waylan, 1999) alter the metabolism of α-tocopherol (a potent antioxidant). Thus, CLA may not exhibit antioxidant properties in an *in vitro* environment, but *in vivo* CLA seems to alter α-tocopherol metabolism in a manner that could imply antioxidant properties for either CLA or MTO. Reduced lipid peroxidation in the mammary gland (but not the liver) was observed after feeding CLA (Ip *et al.*, 1991); however, less CLA was needed to inhibit lipid peroxidation maximally (0.25%) than to inhibit tumor growth maximally (1.00%) in the mammary gland. Another consideration with regard to CLA and cancer modulation revolves around the supplementation period. The inhibitory effect of CLA on the proliferation of cancer cells is only temporary; cells begin to multiply again upon CLA withdrawal (Durgam and Fernandes, 1997). This study further concluded that CLA blocks the division of the cancer cell at the G0/G1 phase of the cell cycle, thus keeping cancerous cells in the cell cycle longer and reducing their ability to multiply. All this evidence indicates that CLA is a potent modulator of carcinogenesis and acts on a wide array of cancer cell lines through improvements in immune status, potential antioxidant capabilities and a direct effect on cancerous cells. Additionally, if CLA is to be used in patients with cancer, the supplementation must be continuous.

Conjugated linoleic acid and diabetes

Conjugated linoleic acid has been linked directly to increased insulin sensitivity, normalized glucose tolerance, improved hyperinsulinemia and lowered levels of circulating free fatty acids in the prediabetic Zucker fatty rat (Houseknecht *et al.*, 1998b). These responses were attributed to the activation of peroxisome proliferator-activated receptor-γ ($PPAR_\gamma$). Conjugated linoleic acid was shown to activate the $PPAR_\gamma$ reporter gene dose-dependently. The activation of PPARs, particularly $PPAR_\gamma$, has been linked to a lowered level of circulating glucose and improved insulin function in animals and humans (Houseknecht *et al.*, 1998b). Increased incorporation of circulating glucose into adipocytes has also been linked to CLA (Satory and Smith, 1999). The retroperitoneal depot has been identified as the fat depot most sensitive to reduction by CLA (West *et al.*, 1998). This fact has important implications for human health because the visceral fat mass is correlated most closely with insulin resistance in non-insulin-dependent diabetes mellitus (Bjorntorp, 1999). These findings suggest that CLA has potential in the management of type II diabetes mellitus. CLA is also being investigated as a modulator of the typically increased free-radical activity in type I diabetes mellitus (Collier *et al.*, 1998) and of the apolipoprotein composition of polyunsaturated fatty acids in type II diabetes mellitus (Singh *et al.*, 19920.

Conjugated linoleic acid and food safety

Food safety is a growing concern, and CLA may offer potential as a food-borne safety factor. Extrapolation from rat data indicates that a 70-kg person needs to consume about 3.5 g of CLA daily to benefit from its cancer-prevention attributes (Ip *et al.*, 1994b). However, the estimated daily CLA consumption from a typical Western diet is only about 1 g (Ha *et al.*, 1989). Thus, the natural consumption of adequate amounts of CLA may not be plausible and dietary supplementation is probably necessary. Heterocyclic amines, which are dietary mutagens and include 2-amino-3-methylimidazo[4,5-*f*]quinoline and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine, have been negated by CLA (Schut *et al.*, 1997). Therefore, the use of CLA may be a practical approach to counteracting these dietary mutagens associated with the process of cooking. This approach also could be used in further-processed meats such as bacon, in which nitrosamine formation is still a concern. The use of CLA to improve food safety should be particularly appealing to people who desire a natural (i.e. food-based) approach to cancer prevention and modulation without drastic alteration of their diet (Ip *et al.*, 1994b). Currently, dietary CLA supplements are available for human consumption, but no source of CLA (including MTO) is approved for use in any livestock diet.

Uses of conjugated linoleic acid in animal production

With the advent of commercial processing facilities for CLA and MTO, their use in poultry and pig studies is now feasible. Although CLA can affect virtually all areas of production, the primary focus has been on improvements in growth performance [ADG, ADFI, and the growth:feed intake ratio (G:F ratio)], body composition and carcass

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leanness (backfat and muscling), belly firmness and meat quality. Three sources of CLA (two from sunflower oil processing and MTO) and a range of supplementation periods from 45 (Dugan *et al*., 1997) to 93 (Thiel *et al*., 1998) days have been used in pig studies. Inclusion rates of dietary CLA and MTO have ranged from 0.12 (Thiel *et al*., 1998) to 2% (Dugan *et al*., 1997). Dose titration studies have been conducted with CLA (Thiel *et al*., 1998), but linear improvements were observed up to the highest level fed (1%), whereas a dose titration study with MTO (O'Quinn *et al*., 2000a) yielded an optimal dietary inclusion level of 0.50% to maximize carcass leanness. This optimal level is in general agreement with a wealth of data from rats suggesting this level for CLA (Chin *et al*., 1994; Belury *et al*., 1997; Park *et al*., 1997, 1999a). Until the optimal dose level of CLA for pigs is elucidated, 1% dietary inclusion (Thiel *et al*., 1998) will probably continue to be used for the tested source of CLA derived from sunflower oil.

Average daily gain

Conjugated linoleic acid has been shown to improve the body weight gain of rat pups during lactation and after weaning (Chin *et al*., 1994). Thus, it appears to exert growth-promoting effects in the offspring when only the dam is supplemented. However, CLA in mice (Bellury *et al*., 1997; West *et al*., 1998) and MTO in rats (O'Quinn *et al*., 1999) have also been reported to reduce weight gain. CLA has been reported to increase (Thiel *et al*., 1998), decrease (Eggert *et al*., 1999c; O'Quinn *et al*., 2000a), and to have no effect (Dugan *et al*., 1997) on ADG in pigs. Decreased ADG has not been observed in pigs receiving MTO supplements, but improvements (O'Quinn *et al*., 1999b, c, 2000a) and no effect (Woodworth *et al*., 1999; O'Quinn *et al*., 2000a, b) have been reported. Reasons for the sporadic improvements in growth in animals receiving CLA or MTO supplementation may be related to differences in health status, environment, age, sex, genetic line, diet composition, prior nutrition, supplementation period, or a combination or interaction of any of these factors.

Average daily feed intake

Reduced feed intake has been reported consistently with CLA supplementation (Dugan *et al*., 1997; Park *et al*., 1997; Eggert *et al*., 1999c; West *et al*., 1998; O'Quinn *et al*., 2000a). No cases of feed refusal have been observed with MTO, and it has been reported to increase ADFI in some trials with pigs (O'Quinn *et al*., 1999b, 2000b). The increased ADFI (O'Quinn *et al*., 1999b) occurred even when supplementary soybean oil (2–3%) was present, indicating that the effects of CLAs are not affected by the presence of additional dietary fats (Haumann, 1996). Additionally, MTO prevented an expected decline in ADFI (without affecting ADG or the G:F ratio) when 6% poultry fat or choice white grease was also present in the diet (Woodworth *et al*., 1999). Several studies have concluded that the reduced feed intake that occurs with CLA supplementation is not large enough to account for the improvement in body composition (Park *et al*., 1997; West *et al*., 1998). Others have postulated that CLA may have palatability or postingestive effects that lead to food aversion, or that CLA could modulate appetite without affecting the animal adversely (West *et al*., 1998). This possible modulation of appetite could be related to the ability of CLA to alter energy metabolism. Recently, CLA was shown to activate dose-dependently the $PPAR_{\gamma}$ gene (Hauseknecht *et al*., 1998b), which partially controls transcription of the leptin gene Houseknecht *et al*., 1998a). Leptin, discovered in 1994 (Zhang *et al*., 1994), is secreted primarily by white adipocytes (Houseknecht *et al*., 1998a), and insulin stimulates its release. Leptin reduces body weight, feed intake and plasma insulin and glucose levels by acting on the hypothalamus to inhibit the release of neuropeptide Y (a potent stimulator of appetite and intake) (Wolf, 1996). Thus, the reduced feed intake that is generally associated with CLA supplementation may be linked to an increase in leptin expression elicited by the CLA. However, this argument may not hold true for MTO, as evidenced by increased feed intake when it is used as a supplement.

Feed efficiency

Improvements in feed utilization efficiency (G:F ratio) have been observed with both CLA (Chin *et al*., 1994; Dugan *et al*., 1997; Thiel *et al*., 1998) and MTO (O'Quinn *et al*., 2000b; O'Quinn PR, Waylan AT and Nelssen JL, *et al*., submitted for publication). Interestingly, improvements in individual components of growth performance from CLA or MTO supplementation are not necessarily coupled or related to improvements in other components. Improvements in carcass leanness are not necessarily linked to growth performance and have not always been associated with improvements in meat quality.

Body composition

The most consistent response to CLA or MTO supplementation has been an improvement in body composition [increased lean mass and(or) reduced adiposity] regardless of the wide variety of experimental methods and animal models used. Reductions in adiposity have been observed in mice (Park *et al*., 1995, 1997; West *et al*., 1998), chickens (Park *et al*., 1995), rats (Sisk *et al*., 1998) and pigs (Dugan *et al*., 1997; Thiel *et al*., 1998). Additionally, MTO has been observed to reduce adiposity in rats (O'Quinn *et al*., 1999) and pigs

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(O'Quinn *et al.*, 1999b, 2000a–c). Increased muscling (longissimus muscle area) has been noted in pigs fed both CLA (Dugan *et al.*, 1997) and MTO (O'Quinn *et al.*, 2000a), but less frequently than the decreases in adiposity. The increases in lean mass from CLA or MTO supplementation in laboratory animals are also variable. Lean body mass in mice fed CLA (Park *et al.*, 1997) was increased from 5 to 14% in one study, but decreased in another study (West *et al.*, 1998). Modified tall oil increased lean mass in ovariectomized rats by about 5% whereas it reduced total body fat by about 21% (O'Quinn *et al.*, 1999). Clearly, the primary improvements in body composition from CLA or MTO supplementation are through reductions in adiposity rather than improvements in lean mass. Feeding CLA is reported to increase norepinephrine-induced lipolysis, hormone-sensitive lipase activity and the activity of total carnitine palmitoyltransferase (Pariza *et al.*, 1997). Additionally, CLA reduced lipoprotein lipase activity while increasing lipolysis, and stimulated fatty acid ß-oxidation in skeletal muscle and fat pad, but not liver (Park *et al.*, 1997). These results have been questioned, and it has been suggested alternatively that, when given during a period(s) of hyperplastic growth, CLA depresses body fat accumulation by reducing the number of preadipocytes (Satory and Smith, 1999). These researchers observed increased adipocyte size and lipid content in response to CLA supplementation, which would be unlikely if lipolysis were favored. Reductions in adipocyte volume also were linked to reductions in adipose tissue mass by CLA (Sisk *et al.*, 1998). Differing methods and CLA concentrations *in vitro* have undoubtedly contributed to the confusion over the exact mode(s) of action of CLA in reducing adiposity. Increased metabolic rates also were observed in mice fed CLA (Wes *et al.*, 1998). Therefore, a combination of these findings probably contributes to reduced adiposity with CLA supplementation.

Belly firmness

Soft bellies that require additional labor and time inputs by meat processors are costly in the pig industry. Soft bellies often result when pigs are fed diets high in fat or oil content or when supplementary fats and oils are of low quality. Feeding CLA (Thiel *et al.*, 1998; Eggert *et al.*, 1999c) and MTO (Woodworth *et al.*, 1999; O'Quinn *et al.*, 2000a; O'Quinn PR, Waylan AT, Nelssen JL, *et al.*, submitted for publication; O'Quinn PR, Waylan AT, Nelssen JL, *et al.*, submitted for publication) to pigs has dramatically increased the firmness of the bellies by as much as 26% compared with those of non-supplemented pigs, regardless of other factors, such as sex, slaughter weight and the level of supplementary dietary fat. As previously reported (Eggert *et al.*, 1999; O'Quinn *et al.*, 2000a), the cause of the increase in belly firmness is a saturating effect of the fatty acids present in the adipose tissue. Feeding MTO or CLA reduced the oleic acid content (the most predominant fatty acid in pork fat) by nearly 20% (O'Quinn *et al.*, 2000a). This resulted in a saturated:unsaturated fat ratio of 0.91:1 compared with 0.65:1 for non-supplemented contemporaries. A minor contributor to belly firmness from CLA supplementation could be the lipid-filling effect noted earlier (Satory and Smith, 1999). Increases in total saturated fatty acids have also been observed in rats fed CLA, leading to the suggestion that CLA inhibits liver Δ9-desaturase activity (Lee *et al.*, 1995; Li and Watkins, 1998). With the proper use of CLA or MTO, some producers may be able to obtain the benefits of increasing dietary fat without the worry of reducing belly firmness or bacon sliceability.

Meat quality

Meat quality is also a growing concern in the pig industry and is especially important with processors that are vertically integrated. Typically, feed additives that improve carcass leanness in pigs do so at the expense of marbling or shear force (Goodband *et al.*, 1993) or some other measure(s) of meat quality. This does not appear to hold true with CLA or MTO supplementation in pigs. Conjugated linoleic acid (Dugan *et al.*, 1999; Wiegand *et al.*, 1999) and MTO (O'Quinn PR, Nelssen JL, Unruh JA, *et al.*, submitted for publication) have been shown to increase intramuscular marbling. Tissue lipid enrichment also has been noted in mice (Belury *et al.*, 1997) and rats (O'Quinn *et al.*, 1999) fed CLA and MTO, respectively. Modified tall oil does not affect the ultimate pH or sensory characteristics of the longissimus muscle of pigs, including shear force and taste panel evaluations. When fed in conjunction with elevated levels of vitamin E (Waylan, 1999), MTO helps to improve meat quality by extending display color stability and reducing oxidative deterioration. Numerous reports have evaluated the effects of CLA on pork quality (Dugan *et al.*, 1999; Eggert *et al.*, 1999a, b; Larsen *et al.*, 1999; Sparks *et al.*, 1999; Thiel-Cooper *et al.*, 1999; Wiegand *et al.*, 1999; O'Quinn *et al.*, 2000a). With a few minor exceptions, the general conclusion was that CLA does not have any deleterious effect on pork quality. These exceptions included lower longissimus a* values (redness) (O'Quinn *et al.*, 2000a) and higher ham L* values (lightness) (Larsen *et al.*, 1999). Feeding MTO has also been found to have no effect on water-holding capacity (O'Quinn *et al.*, 2000b), but did reduce longissimus b* values (yellowness) (O'Quinn *et al.*, 2000a). This reduction in off-color could be related to the ability of MTO to act with α-tocopherol to stabilize intramuscular fat (Waylan, 1999). These data imply that either CLA or MTO can be used to improve carcass leanness in pigs without damaging pork quality. Some measures of meat quality can be improved with CLA or MTO alone or in combination with other feed additives, such as vitamin E.

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Other considerations

In addition to the studies mentioned above, CLA has been investigated in relation to a host of other research areas. It has been proposed that CLA modulates cancer cell growth by interfering with the hormone (estrogen)-regulated mitogenic pathway (Durgam and Fernandes, 1997). Conjugated linoleic acid has also been studied for its involvement in reducing atherosclerosis (Lee *et al*., 1994; Nicolosi *et al*., 1997) and for a possible role in bone formation and resorption (Li and Watkins, 1998). A recent study investigated the effects of CLA on the basal metabolism of mice in a respiratory chamber (West *et al*., 1998). Though the effects of MTO are less well studied, it did not affect bone mineral content or bone mineral density in ovariectomized rats (O'Quinn *et al*., 1999). The role of CLA in autoimmune disorders and asthma has also been investigated (Cook and Pariza, 1998). Two studies have considered the effects of MTO on fat color, and in both instances it appeared to increase the a* values of the fat without affecting the lightness (L*) values (O'Quinn *et al*., 1999b, c). Though a* values indicate redness, these increases could have been browning effects caused by unsaponifiable matter in MTO that is incorporated into the fat and causes slight discoloration. However, more time and research will be needed to add credibility to or disprove the roles of CLA and MTO in any of these lightly studied areas.

Future goals of research on conjugated linoleic acid

Obviously, CLA and MTO affect a large range of biological systems. Many of the responses elicited by CLA are interconnected. For example, improvements in immune status could affect potential carcinogenesis or other health issues such as diabetes. Future research efforts will need to focus on defining optimal periods of supplementation with CLA and MTO for pigs and poultry and determining if their supplementation can improve color stability and reduce oxidative deterioration of beef. The causes of differences in growth performance of pigs fed CLA and MTO need to be elucidated. For humans, a decision should be made about dietary CLA supplementation (for example, should a healthy person take CLA?). Differences in methods need to be resolved; at least 10 different rodent models have been used, and a variety of sources of CLA, dose levels and supplementation periods have been employed in both rodent and livestock studies. Finally, the toxicology studies necessary for FDA approval for the use of CLA or MTO as an animal feed additive must be conducted. The use of either one as an animal or human feed/food additive offers tremendous potential for improvements in aspects of the quality of life for humans and economic gains for livestock producers.

Conclusion

Sources of conjugated linoleic acid (such as those derived from sunflower oil or tall oil) offer promise in all areas of animal agriculture, ranging from improvements in growth performance and carcass leanness to increasing the color stability of fresh meat and the potential modulation of food-borne contaminants. Conjugated linoleic acid also will affect human health and nutrition because of its ability to modulate carcinogenesis, whether directly or through ingested foods.

Key challenges for the widespread acceptance of conjugated linoleic acid in animal production will revolve around the ability of manufacturers to obtain FDA approval for its use as an animal feed/food additive and to produce large quantities at an economically feasible price. Additionally, the source of conjugated linoleic acid being used must be known because of the differences in performance that result from the different types available. However, these challenges are attainable, especially when weighed against the multiple benefits gained from feeding conjugated linoleic acids to livestock.

Abbreviations

CLA, conjugated linoleic acid; MTO, modified tall oil; PGE_2, prostaglandin E_2; TNF-α, tumor necrosis factor-α.

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