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# Biological Activities of Conjugated Linoleic Acids and Designer Eggs

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Recent investigations suggest that conjugated linoleic acid (CLA) isomers possess anticarcinogenic properties, including inhibition of forestomach cancer in mice, suppression of mammary tumours in rats and inhibition of cancer cell proliferation. CLA may also function as a repartition agent in growing animals. For example, when young rodents, pigs and broiler chicks were fed CLA, they demonstrated improved feed efficiency and reduced body fat. Few studies, however, describe the effects of CLA on egg production and fatty acid composition of yolk lipids.

The purpose of the present investigation was to measure the effects of CLA supplementation on egg yolk composition in laying hens. Forty Single Comb White Leghorn hens were divided into four groups of ten hens, and egg production and yolk fatty acid composition measured over 4 months. Group 1 served as the control (not supplemented with oil), group 2 received 1 g of CLA every other day, group 3 received 1 g of CLA every fourth day, and group 4 was sham-supplemented (given 1 g of safflower oil) every other day. Hen egg and yolk weights increased during the supplementation period but were not significantly affected by the oral lipid treatments. The results of the gas chromatographic analysis indicated that CLA was incorporated successfully into the yolk lipids. Egg yolks contained all of the CLA isomers present in the human supplement [9,11 (*trans*-, *cis*- and *cis*-, *trans*-), 10,12 (*trans*-, *cis*-) and 9,11 and 10,12 both *cis*-, *trans*-isomers]. Upon analysis, hen blood lipids showed no changes in very low-density lipoprotein, triglyceride, phospholipid, cholesterol ester and free cholesterol levels due to the lipid treatments. The CLA concentration in egg yolk was positively correlated with the frequency of supplementation. Although egg yolk fatty acid composition was altered by supplementation, blood lipids and egg production were not influenced by CLA.

## Introduction

### Chemistry of conjugated linoleic acids (CLAs)

CLAs are positional and geometric isomers of conjugated octadecadienoic acids that occur naturally in several foods, but their concentration is highest in dairy and beef products. The CLA isomers lack a methylene group separating the double bonds located at the Δ-9 and Δ-12 positions of the essential fatty acid linoleic acid. The growing body of literature on CLAs suggests that these isomeric conjugated fatty acids promote beneficial health and biological effects (Ip *et al.*, 1994, 1996; Lee *et al.*, 1994; Decker, 1995).

The positional isomers of CLA include 7,9-, 8,10-, 9,11-, 10,12- and 11,13-conjugated octadecadienoic acids (counting from the carboxyl end of the molecule). Each of the aforementioned positional conjugated diene isomers can occur in the following geometric configurations: *cis*-, *trans*-; *trans*-, *cis*-; *cis*-, *cis*-; and *trans*-, *trans*- (Haumann, 1996; Sehat *et al.*, 1998). The most common CLA isomer found in natural products is *cis*-9, *trans*-11-octadecadienoic acid, which is now proposed to be named 'rumenic acid' (Kramer *et al.*, 1998a).

CLA isomers possess many unique biological activities compared with linoleic acid. The research on CLAs indicates anticarcinogenic, antiatherosclerotic, antioxidative, immunomodulative and antibacterial effects (Scimeca *et al.*, 1994; Decker, 1995; Haumann, 1996; Parodi, 1996; Sugano *et al.*, 1998). One of the earliest experiments on CLAs indicated that these fatty acids, isolated from extracts of grilled ground beef, exhibited anticarcinogenic activity against chemically induced skin cancer in mice (Ha *et al.*, 1987). Recent experiments on prostate cancer cell lines demonstrated that CLA isomers are incorporated into cell lipids and that CLAs compared with linoleic acid decreased cell proliferation (Cornell *et al.*, 1997). Furthermore, CLAs were found to reduce prostaglandin $E_2$ concentrations in rat serum, spleen (Sugano *et al.*, 1997) and *ex vivo* bone organ culture (Li and Watkins, 1998).

### Sources of CLAs

CLAs are found naturally in a wide variety of food products. Some of the foods are beef, lamb, poultry, seafood, cheese, butter, milk and vegetable oils (Ip, 1994). Fats and meats from ruminant species are the richest natural sources of CLAs. Lamb, veal and beef contain from 2.7 to 5.6 mg of CLA $g^{-1}$ of fat (Haumann, 1996). Cheese and milk fat have about 3–6 mg of CLA $g^{-1}$ of fat (Ip, 1994). The linoleic acid present in the diets of grazing animals is converted to CLA by an isomerase which is released by ruminal bacteria (*Butyrivibrio fibrisolvens*) (Chin *et al.*, 1992) as a part of the biohydrogenation process (Bartlet and Chapman, 1961). CLA may also be produced from linoleic acid in the colon by microorganisms of conventional rats since no CLA was detected in the faeces of germ-free rats given the same diet (Chin *et al.*, 1994a). In most cases, the *cis*-9, *trans*-11 isomer is the predominant isomeric form of CLA found naturally, except for vegetable oils which can contain several other isomers. Estimates of CLA intake range from 1.5 to 0.3 g per person $day^{-1}$ and

appear to be dependent on gender and the intake of animal and vegetable foods (Fritsche and Steinhart, 1998).

Besides the natural sources, CLAs are produced synthetically by alkali isomerization of linoleic acid (Haumann, 1996). The synthetic sources of CLA are prepared from linoleic acid or vegetable oils (sunflower and safflower) which are rich in linoleic acid (Ha *et al.*, 1990; Christie *et al.*, 1997). Many commercial CLA products are available as supplements, but their composition may be variable (Christie *et al.*, 1997).

## Biological activities of CLA

### Growth

Several investigators have documented that feeding CLAs improved feed efficiency and reduced body fat deposition in growing pigs, rats, mice, rabbits and chickens (Cook *et al.*, 1993; Chin *et al.*, 1994b; Haumann, 1996; Parodi, 1996; Park *et al.*, 1997; Sugano *et al.*, 1997; Li and Watkins, 1998). Nutritional studies with CLA isomers showed that these fatty acids are incorporated into different organs, and that both neutral and polar lipid fractions of animal (Belury and Kempa-Steczko, 1997; Sebedio *et al.*, 1997; Sugano *et al.*, 1997; Kramer *et al.*, 1998b; Li and Watkins, 1998) and human tissues (Fritsche *et al.*, 1997) were enriched. Moreover, CLAs may affect the fatty acid composition and content of rodent tissues and cultured cells (Li and Watkins, 1998). These experiments suggest that the incorporation of CLAs may be dose and time dependent in animals, humans and cell culture models. For example, Huang *et al.* (1994) gave healthy men CLA in cheese and found that the plasma CLA concentration increased significantly with cheese consumption. Although the data are limited on the toxicity of CLA, a recent study by Scimeca (1998) indicated no treatment-related effects of CLA on histopathological and haematological analyses of blood from rats.

### Carcinogenesis

Potential anticarcinogenic effects of CLA have been demonstrated by both *in vivo* and *in vitro* studies. Pariza *et al.* (1983) tested the inhibitory effects of crude CLA extracts from fried ground beef against two mutagens, 2-amino-3-methylimidazo[4,5-*f*]quinoline and 2-aminofluorene mediated by rat liver S-9, in normal, phenobarbital- or aroclor-treated rats. In this study, the CLA extract showed an inhibitory effect on chemically induced mutagenesis.

Shultz *et al.* (1992a) investigated the effect of CLA on human MCF-7 breast cancer cell growth. Cancer cells were enriched with varying concentrations of linoleic acid and CLA ($1.7–7.1 \times 10^{-5}$ M) for 12 days. While linoleic acid initially stimulated cancer cell growth at concentrations of $3.5–7.1 \times 10^{-5}$ M, CLA inhibited cell growth after 8–12 days of incubation at the same linoleic acid concentrations. In addition, cytotoxicity for MCF-7 cells was greater with CLA than that with linoleic acid. Shultz *et al.* (1992b) also observed that CLA inhibited the growth of HT29 colon cancer cells; however, conflicting results on the effects of C18 fatty acid isomers on cancer cells have been reported (DesBordes and Lea, 1995). In 1997, Liu and Belury reported that CLA

significantly decreased ornithine decarboxylase activity, which is a hallmark event of tumour promotion, in the cultured keratinocyte cell line HEL-30.

There is convincing evidence that CLA isomers inhibit carcinogenesis in animal models (Haumann, 1996). Ip and co-workers (1991, 1994, 1995, 1996) showed that dietary supplementation with CLA (free acid at 1% by weight) resulted in protective effects against mammary carcinogenesis in rats. Moreover, Ip *et al.* (1991) found that CLA inhibited the development of mammary tumours induced by a high dose of dimethylbenz(*a*)anthracene in rats fed synthetically prepared CLA (0.5, 1.0 and 1.5% of the diet) for 24 weeks. The number of mammary adenocarcinomas was reduced by 32, 56 and 60%, respectively, compared with the controls given the AIN-76A basal diet. The final tumour incidence and cumulative tumour weight also decreased in rats fed the diet containing CLA. The response observed in these experiments might be dose dependent at levels of 0.5–1% CLA. The *cis*-9, *trans*-11 isomer of CLA was detected in the phospholipid fraction of liver and mammary tumours of rats, and the incorporation of *cis*-9, *trans*-11 increased with dietary intake. In addition, CLA was observed to inhibit lipid peroxidation in the mammary gland but not in the liver.

Rats at a similar age were given lower doses of dietary CLA (0.05, 0.1, 0.25 and 0.5%) for 5 weeks, and a dose-dependent inhibitory effect in chemically induced mammary tumour formation was observed for all doses (Ip *et al.*, 1994). The anticarcinogenic activity of CLA in the methylnitrosourea model suggests that CLA may have a direct modulating effect on susceptibility of the target organ to neoplastic transformation. However, CLA also showed inhibitory effects on proliferative activity of the mammary gland. More recent studies indicate that the free acid and triacylglycerol forms are essentially identical in providing anticancer activity in rats (Ip *et al.*, 1995). Furthermore, a continuous dietary supplementation with CLA was required for maximal inhibition of tumour formation.

The anticarcinogenic activity of CLA has been confirmed in other animal models. For example, CLA was reported to suppress initiation of skin carcinogenesis induced by 7,12-dimethylbenz(*a*)anthracene (Parodi, 1996) and phorbol ester skin promotion in mice (Liu and Belury, 1997). Inhibition of benzo(*a*)pyrene-induced forestomach neoplasia was observed in mice given synthetic CLA by gavage (Ha *et al.*, 1990). Four and two days before administration of benzo(*a*)pyrene, mice were given one of the treatments four times (0.1 ml of CLA, 0.1 ml of linoleic acid or 0.1 ml of 0.85% saline as a sham control). Compared with the control, a 50% reduction in the neoplasms was observed in animals given CLA. Only the *cis*-9, *trans*-11 isomer of CLA was detected in forestomach phospholipids in this study.

Protective effects of CLA on colon carcinogenesis induced by 2-amino-3-methylimidazo[4,5-*f*]quinoline were investigated using male F344 rats (Liew *et al.*, 1995). The 2-amino-3-methylimidazo[4,5-*f*]quinoline is a mutagenic heterocyclic amine that develops naturally in cooked meat and fish but requires metabolic activation to be carcinogenic. CLA resulted in a significant inhibition of DNA–IQ formation at a dietary level of 0.5% by weight.

Ip *et al.* (1996) and Ip and Scimeca (1997) also investigated the anticarcinogenic mechanism of CLA by studying the interactions between CLA and other fat sources. The anticarcinogenic activity of CLA was evaluated in diets containing fat levels of 10, 13.3, 16.7 and 20% by weight, and 20% fat from either corn oil or lard (Ip *et al.*, 1996). The results indicated that mammary cancer prevention produced by 1% CLA was not influenced by the level or type of fat in the diet. Unfortunately, these studies did not consider the influence of *n*-3 polyunsaturated fatty acids which are known to be protective against carcinogenesis (Rose, 1997). Ip *et al.* (1997) further reported that the efficacy of the anticarcinogenic activity of CLA was not altered by dietary linoleic acid intake. Interestingly, the accumulation of CLA in mammary tissue was found to be dose dependent for certain dietary CLA levels, and that the dietary linoleic acid level did not affect incorporation of CLA into either neutral or polar lipids.

### Atherosclerosis

CLA has also exhibited antiatherosclerotic activity in both the rabbit and hamster (Lee *et al.*, 1994; Nicolosi *et al.*, 1997). Hamsters were given a hypercholesterolaemic diet and a supplement of 0.06, 0.11 and 1.1% CLA (Nicolosi *et al.*, 1997). CLA significantly reduced signs of early atherosclerosis, and reduced the total serum cholesterol, low-density lipoprotein (LDL) cholesterol and triacylglycerols without influencing high-density lipoprotein (HDL) cholesterol. Cholesterol-lowering effects of CLA were observed and confirmed in a rabbit feeding study by Lee *et al.* (1994). Rabbits were given a semi-synthetic diet containing 14% fat and 0.1% cholesterol with a supplement of 0.5 g of CLA $day^{-1}$ over 12 weeks. The CLA-supplemented rabbits had markedly lower levels of total serum cholesterol, LDL cholesterol, and triacylglycerols compared with those not given CLA. Moreover, the cholesterol ratios of LDL to HDL and the total serum to HDL were significantly reduced in the CLA-supplemented group. Examination of the aorta revealed less atherosclerotic plaque formation in rabbits given CLA. The antiatherosclerotic property of CLA was comparable with that of linoleic acid in hamsters (Haumann, 1996). Reductions in cholesterol levels by either linoleic acid or CLA were similar, i.e. about 20%, when linoleic acid or CLA was provided at 2% of dietary calories. The reduction in symptoms of early atherogenesis, however, was approximately three times greater with the CLA diet compared with the linoleic acid diet. Although antiatherosclerotic activity of CLA has been reported, and despite a possible relationship to antioxidant properties, the exact mechanism for its action has not been elucidated.

### Antibacterial

The potassium salts of CLA were found to have antibacterial activity against *Listeria monocytogenes* at a concentration of 50 $\mu g\ ml^{-1}$ (Wang and Johnson, 1992). CLA showed higher bactericidal activity in brain–heart infusion broth at pH 5 than at pH 6. Potassium salts of CLA exhibited a bacteriostatic activity, thereby prolonging the lag phase in both whole milk and skimmed milk at 4°C. The length of the lag phase was proportional to the concentration of the CLA salts in skimmed milk at 25°C. Other fatty acids tested were not as

effective in skimmed or whole milk, although some of them were bactericidal in brain–heart infusion broth.

## Antioxidant

Ha *et al.* (1990) reported that CLA acted as an antioxidant *in vitro*. The antioxidant activity of CLA isomers was evaluated using the thiocyanate method and compared with known antioxidants, including α-tocopherol, ascorbic acid and butylated hydroxytoluene (BHT). The experiment was conducted by adding ferrous ammonium sulphate and thiocyanate into an aqueous linoleic acid solution, followed by measuring the degree of linoleic acid oxidation (peroxide value). CLA showed more powerful antioxidative activity than α-tocopherol, comparable with that for BHT. The above observation was supported by Ip *et al.* (1991). The lipid peroxidation products in rats fed CLA were quantified by measuring endogenous thiobarbituric acid-reactive substances in liver and mammary gland. The results showed that CLA reduced lipid oxidation in mammary gland but not in liver. Maximal antioxidant activity was observed with only 0.25% CLA in the diet, while the greatest anticarcinogenic effect was obtained at a dietary CLA level of 1%. In understanding the metabolism of CLA, one recent study (Yurawecz *et al.*, 1995) suggests that CLA is converted into furan fatty acids during oxidation and should be considered as an endogenous source of furan fatty acids in biological systems.

The effects of CLA on the activities of protein kinase C (Benjamin *et al.*, 1992) and phospholipase C (Bonordon *et al.*, 1993) might aid in understanding other biological actions of these fatty acids that relate to antioxidant properties. These experiments suggest that CLA may alter signal transduction and, since the redox (oxidation–reduction) state has been related to signal transduction in biological systems, CLA may reduce messenger molecules (reactive oxygen species or cytokines) involved in cell–cell signalling. Oxidative stress is associated with many degenerative diseases, and the present knowledge might support a role for CLA in protection against this condition.

## Immunomodulation

CLA isomers appear to act as immunomodulating agents and they may protect against pathogenic microorganisms to aid in reducing allergic reactions (Parodi, 1996; Sugano *et al.*, 1998). In an investigation on immune cell function, Turek *et al.* (1998) found that CLA could influence cytokine production by peritoneal macrophages in rats given diets varying in *n*-6 and *n*-3 fatty acids. The study revealed that CLA reduced basal and lipopolysaccharide-induced levels of interleukin-6 by macrophages in rats given soybean oil, and reduced basal levels of tumour necrosis factor production by macrophages in rats given a high *n*-6 or *n*-3 fatty acid diet.

Besides the effects on cytokines, CLA isomers were found to reduce tissue prostaglandin $E_2$ ($PGE_2$) levels in rats (Sugano *et al.*, 1997; Li and Watkins, 1998). Other studies suggest that CLA can influence immune cell function. For example, Wong *et al.* (1997) reported that CLA might modulate immune defence, including lymphocyte proliferation in mice, and Sugano *et al.* (1998) observed that CLA altered chemical mediators and immunoglobulins (Igs) in

rats. Although these experiments showed that CLA did not influence histamine release, it did reduce the amount of leukotriene $B_4$ from peritoneal exudate cells, spleen and lung. Furthermore, the levels of IgA, IgG and IgM increased in spleen and mesenteric lymph node lymphocytes, but the IgE level decreased in rats given a 1% dietary level of CLA. Some research suggests that CLA may stimulate porcine lymphocyte blastogenesis and, in a study with rats, Cook *et al.* (1993) observed that CLA increased mitogen response and macrophage phagocytosis in rats. In support of the previous investigation, Miller *et al.* (1994) reported that spleen lymphocyte blastogenesis was enhanced in rats given CLA.

### Bone

In a recent investigation on bone modelling, Watkins *et al.* (1997) reported that milk fat stimulated bone formation rate in growing chicks possibly by modulating *ex vivo* $PGE_2$ production in bone. Since milk fat is a primary dietary source of CLA, having a concentration up to 30 mg of CLA $g^{-1}$ fat (Parodi, 1996), the positive effect of milk fat on bone formation might be related to modulating $PGE_2$ production. With regard to bone metabolism, $PGE_2$ is recognized to be a mediator of both bone formation and bone resorption *in vivo* (Marks and Miller, 1993), and moderate levels of this prostanoid favour bone formation. The recent work of Li and Watkins (1998) demonstrated that CLA at 1% of the diet reduced *ex vivo* $PGE_2$ production in bone of rats independently of the amount of dietary *n*-6 or *n*-3 fatty acids. At present, no study has been published that describes how CLA supplements affect bone modelling or remodelling in animals or humans.

## Designer Eggs

For years, scientists and health professionals have been investigating and debating the effects of foods, nutrients and diet on promoting health and reducing disease. Many nutritionists support and recommend a low fat, high fibre diet to healthy people interested in dietary prevention of chronic disease. As the press disseminates information from nutrition/health studies, often the results are inadequately presented and difficult for the general public to interpret. Once scientific criteria are established for developing health claims for functional/ designed foods, the relationships between nutrients and disease will become clear (Clydesdale, 1997). Moreover, the curiosity in self-medication, the explosion in the elderly population and rising health care costs fuel the interest in foods and their potential health benefits. Because of the intense interest in the relationship between diet and health, much attention has been paid to manipulating foods to promote health. The term 'designer foods' emerged some years ago to describe a food tailored to contain specific concentrations and proportions of nutrients critical to good health. Other similar terms have been used such as functional foods, nutraceuticals and phytochemical sources. The public interest in new foods that offer health benefits will stimulate opportunities for developing designer foods from poultry products. Although many designer egg products have been marketed around

the world, the continued growth and acceptance of these products will depend on nutritional and health labelling laws.

In January 1993, the Food and Drug Administration (FDA) published regulations implementing the Nutrition Labeling and Education Act of 1990 in the USA. This law requires nutritional labelling information for processed foods, fresh fruit, vegetables and seafood. The Food Safety and Inspection Service published similar regulations pertaining to meat and poultry products. Labels for retail foods contain information on total calories, calories from fat, total fat, saturated fat, cholesterol, sodium, total carbohydrates, dietary fibre, sugars, protein, vitamin A, vitamin C, calcium and iron. Optional nutritional information can include calories from saturated, polyunsaturated and monounsaturated fats, potassium, soluble fibre, insoluble fibre and other vitamins and minerals. These laws bring greater relevance to the issue of linking nutrient composition to health problems since the FDA now allows specific health claims for certain nutrients (Box 14.1). It is not clear yet as to how the public will perceive and utilize this information; however, consumers may respond by making more informed decisions before purchasing foods. As nutrition/health-related research continues, the FDA may allow more health claims for other nutrients.

Cruickshank (1934) was the first to suggest and demonstrate that dietary fats could change the composition of lipids in poultry. More recent attention given to modifying the fatty acid composition of poultry meat and eggs has focused on elevating *n*-3 polyunsaturated fatty acids (PUFAs) because of the health benefits associated with these fatty acids. Since the chicken is a monogastric, much of the dietary fat is assimilated directly with minimal modification (Watkins, 1995). Furthermore, it is well documented that when fishmeal, menhaden oil and flaxseed products are fed, the *n*-3 PUFAs contained in these products are readily incorporated into tissue lipids of broilers and turkeys. In addition, broiler chickens can chain elongate and desaturate dietary sources of α-linolenic acid (18 : 3*n*-3) to form eicosapentaenoic acid (20 : 5*n*-3); however, this process is limited (Watkins, 1995).

The chicken can modulate its tissue concentrations of PUFAs by the types and amounts of fatty acids it consumes (Watkins, 1995) because liver contains desaturation/elongation enzymes to facilitate the formation of PUFAs. In most

**Box 14.1.** Health claims now allowed by the FDA.

- Calcium's link in preventing osteoporosis
- Reduced fat content to reduce the risk of cancer
- Saturated fatty acids and cholesterol links to coronary heart disease
- Fibre in certain fruits and vegetables and cancer prevention and reduced risk of coronary heart disease
- Sodium link to hypertension
- Low fat intakes from diets of fruit and vegetables link to a reduced risk of cancer

practical poultry diets, the essential fatty acid linoleic acid is at a higher concentration than α-linolenic acid. In this case, greater amounts of *n*-6 PUFAs are formed compared with the amounts of *n*-3 PUFAs. When the dietary concentration of 18 : 3*n*-3 increases relative to 18 : 2*n*-6, an elevation of *n*-3 PUFA formation occurs. Feeding sources of *n*-3 PUFAs to poultry increases the carcass concentration of 20 : 5*n*-3 but lowers that of 20 : 4*n*-6. Appreciable amounts of *n*-3 PUFAs also accumulate in egg yolk of hens fed menhaden oil. Commercial interest in feeding flaxseed and flaxseed oil has been exploited to elevate the concentrations of *n*-3 PUFAs in poultry meat and eggs to produce designer foods.

Feeding *trans*-fatty acids from hydrogenated oils can lead to accumulatium in broiler tissues and in egg yolk (Watkins, 1995). Consumption of hydrogenated oil is under intense scrutiny because of its negative effect on blood cholesterol levels which increases the risk of coronary heart disease in humans (Lichtenstein, 1993).

Several common chronic diseases are affected by imbalances in fatty acid metabolism in humans (Watkins *et al*., 1996). An excess of saturated fats is conducive to coronary heart disease and atherosclerosis. Uncontrolled lipid peroxidation causes inflammation, and oxidative damage leading to free radical formation in tissues is believed to contribute to many life-threatening diseases in humans (Ames, 1989). Free radicals are believed to be involved in the development of cardiovascular disease, stroke and certain cancers. The free radicals attack DNA, proteins and PUFAs in cell membranes, and attack on DNA is hypothesized to cause mutagenesis and carcinogenesis. Although the body maintains enzyme systems and levels of natural antioxidants to terminate free radical formation, components of these enzymes and the antioxidants must be supplied continually in the diet. Interest in foods derived from plants has arisen because of their concentrations of phytochemicals that may reduce lipid peroxidation and protect the body from free radical damage (Caragay, 1992). Phytochemicals are abundant in a variety of plants, but those present in soybeans, garlic, cabbage, ginger, liquorice, umbelliferae (carrots and celery) and flax have received a great amount of research attention. Carotenoids, tocopherols, phenolics and flavonoids are examples of plant phytochemicals. Future studies on enriching poultry meat and eggs with antioxidant vitamins and phytochemicals could provide additional opportunities for developing designer foods.

## Enrichment of Layer Egg Yolk Lipids with CLA

Substantial progress has been made in understanding lipoprotein metabolism in the hen and lipid deposition in the developing egg yolk. Furthermore, the deposition of dietary fatty acids into yolk lipids has been studied extensively. Attention given to modifying the fatty acid composition of egg yolks has focused on elevating *n*-3 PUFAs because of the healthy effects associated with these fatty acids. More recently, interest has shifted to research on the health benefits of CLA and their applications to food systems. Since numerous investigations suggest that CLAs possess antiatherosclerotic and anticarcinogenic

properties in addition to reducing body fat in animals, an experiment was conducted to evaluate the effect of feeding CLAs to chicken. The purpose of this study was to measure the effects of CLA supplementation to laying hens on egg yolk lipid composition. Hens were supplemented with a commercial grade soft gel capsule (PharmaNutrients, Tonalin™) containing either CLA or safflower oil. The CLA mixture was a human grade dietary supplement that contained 1 g of CLA isomers encapsulated in the soft gel. CLA capsules contained several isomers of CLA (≈ 55–56% CLA) as free fatty acids.

Forty Single Comb White Leghorn hens (35 weeks of age) were divided into four groups of ten hens, and egg production was observed for 13 weeks. Egg collection continued for another 4 weeks past the supplementation period to monitor CLA content in egg yolk after withdrawal of the treatments. Group 1 served as the control, group 2 received 1 g of CLA every other day, group 3 received 1 g of CLA every fourth day and group 4 was given safflower oil every other day as a sham control. Hens were housed two per cage, and a single cage housing two roosters was utilized for treatment group separation. The laying hens were identified with wingbands and placed in numbered cages.

Ten randomly selected eggs from each group were collected from bi-weekly laying periods throughout the study to determine egg weights and obtain egg yolk samples. The sample shell egg was weighed, shelled and the yolk separated from albumen to determine the yolk weight. Two yolks were combined, mixed thoroughly and an aliquot of 1 g used for lipid extraction. The yolk samples were extracted with chloroform/methanol (2 : 1, v/v) and fatty acid methyl esters (FAME) prepared according to Li and Watkins (1998) for capillary gas chromatographic analysis.

Initial hen weight and egg samples were determined at the onset and completion of the feeding trial, and no significant difference was found between the treatment groups. Egg production averaged seven eggs hen$^{-1}$ week$^{-1}$ and was not altered by the dietary inclusion of CLA. However, hen feed consumption did appear to decrease with increasing CLA supplementation without influencing egg production, thus confirming that CLA could improve feed efficiency. Shell egg weights were greater in hens given CLA; however, yolk weights were unchanged (15–16 g for all groups). Although our results indicate that CLA did not influence yolk consistency, it is not clear if higher levels of supplementation would affect the egg quality.

The results indicated that CLA was incorporated successfully into the yolk lipids. Egg yolks contained the following CLA isomers: 9,11(*trans*-, *cis*- and *cis*-, *trans*-); *trans*-10, *cis*-12; *cis*-, *cis*- (9,11 and 10,12). Total CLA ranged from 0.7 to 1.2% of total fatty acids in the supplemented groups. Upon analysis, hen blood lipids showed no changes in very low-density lipoprotein, triglyceride, phospholipid, cholesterol ester and free cholesterol across the treatment groups. The concentration of CLA in egg yolk was positively correlated with the frequency of supplementation, and CLA content peaked at 5 weeks of dietary supplementation. From these data, it appeared that the *cis*-9, *trans*-11 isomer was incorporated into the yolk lipids more readily, based on the FAME analysis. This result is consistent with the analysis of CLA isomers in foods that naturally contain CLA such as milk, cheese, butter and steak (Fritsche and

Steinheart, 1998; O'Shea *et al.*, 1998; Yurawecz *et al.*, 1998). The fatty acid profile of the enriched yolks exhibited a decrease in the amount of monounsaturates, similar to the findings of reduced tissue monounsaturates in rabbits fed CLA (Lee *et al.*, 1995). The current experiment also revealed that supplementing with CLA and safflower oil elevated the *n*-6 fatty acid content in yolks, which corroborates the findings of March and MacMillan (1989) that linoleic acid feeding to hens elevated the *n*-6 fatty acid content in egg. The total amount of PUFAs was greater in egg yolk of hens given CLA compared with the control, which might suggest that CLA may have been desaturated and elongated (unpublished observations in rat liver; Belury and Kempa-Steczko, 1997). Upon analysis of fatty acids in yolk of stored eggs (4–6°C), the total amount of CLA diminished with increased storage time (6 months). This phenomenon was reported in CLA-containing products such as cheese and milk (Shantha *et al.*, 1995). Eggs from this study contained more CLA (1.25% of the total fatty acids) than other foods such as dairy and beef products (0.29–0.89% of the total fatty acids). This study demonstrated that CLA could be enriched in egg yolk for the development of new designer eggs for the consumer.

## Conclusions

The current popularity of nutrition and interest in the relationships between diet and health provide opportunities for developing designer foods from poultry egg products. As food labelling laws become established, some consumers will respond by making more informed decisions about diet and the foods they select. Their decisions will be influenced by the health claims for nutrients allowed by government agencies. As the list of nutrients grows and as congressional action occurs for nutraceuticals, new designer food products and markets will open for the progressive food industries. Food scientists working with nutritionists should be looking for alternative means to modify the egg to deliver selected nutrients such as *n*-3 PUFAs and CLAs to the consumer.

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