JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Conjugated Linoleic Acids Alter the Fatty Acid Composition and Physical Properties of Egg Yolk and Albumen

BRUCE A. WATKINS,*,† SHULIN FENG,† ANNA K. STROM,† AMY A. DEVITT,† LIANGLI YU,‡ AND YONG LI†

Center for Enhancing Foods to Protect Health, Lipid Chemistry and Molecular Biology Laboratory, Purdue University, West Lafayette, Indiana 47907-2009, and Department of Food Science & Human Nutrition, Colorado State University, Fort Collins, Colorado 80523

Effects of dietary conjugated linoleic acids (CLAs) and docosahexaenoic acid (DHA) on the fatty acid composition of different egg compartments after storage were studied. Four dietary treatments [supplemented with safflower oil (SAFF, control group), DHA, CLAs plus DHA (CAD), and CLAs alone] were administered to Single Comb White Leghorn (SCWL) laying hens. Eggs from the different treatment groups were collected and stored for 10 weeks at 4 °C before analysis. Fatty acids from the yolk (yolk granules and plasma), egg albumen, and vitelline membrane were analyzed by gas chromatography. The yolk of eggs from hens given CLAs had significantly higher amounts of saturated fatty acids, typically 16:0 and 18:0, but lower amounts of polyunsaturated fatty acids (PUFAs) compared to eggs from the control group (SAFF). CLA content was highest in the yolk and present in both neutral and polar lipids, with the greatest concentrations in neutral lipids. DHA was incorporated mainly into yolk polar lipids. Lipids in yolk plasma and granules contained similar amounts of CLAs. The fatty acid compositions of vitelline membrane and egg albumen mirrored that of the egg yolk. CLA supplementation resulted in hard and rubbery yolks when compared to hard-cooked eggs from the control group. This study showed that feeding CLAs to hens led to accumulation of the isomers in polar and neutral lipids of the egg yolk and that these isomers migrated into egg albumen. Because the sensory properties of hard-cooked eggs were negatively affected by the enrichment of a mixture of CLA isomers in this study, further research should be conducted to evaluate how the different isomers alter the properties of egg yolk and albumen so that the quality of designed eggs containing CLAs and DHA can be improved.

KEYWORDS: Conjugated linoleic acids; docosahexaenoic acid; yolk; granule; plasma; designer eggs

INTRODUCTION

Conjugated linoleic acids (CLAs) are purported to provide a number of health benefits. The actions include anticarcinogenic, hypocholesterolemic, and antiartherogenic effects in laboratory animals and cell culture models (*1*−*3*). CLAs have been shown to modulate immune response (*4*, *5*), to decrease body fat (*6*), and to influence bone formation (*7*) in rodents. The recent research focus on CLAs has been describing the actions of specific isomers. For example, the *t*10,*c*12 isomer was found to inhibit the proliferation and differentiation of pre-adipocytes (*8*), which could be partly responsible for the fat-reducing effects of CLAs (*9*, *6*, *10*). Other research suggests that CLAs can directly activate peroxisome proliferator-activated receptor α and β subtypes (*11*), which might indicate a role for CLAs in the direct regulation of gene modulation. Furthermore, the *c*9,*t*11 isomer appears to reduce prostanoid biosynthesis in some tissues (*12*, *13*).

Although potential health benefits have been reported, research into how to deliver desired amounts of CLAs in food animal systems is lacking. Research in our laboratory and others has indicated that CLAs are readily incorporated into egg yolk (*14*−*16*). It is therefore possible to use commercial table eggs as a vehicle food system to enhance human nutrition. However, additional research is needed to ensure that the incorporation of CLAs into eggs will not affect sensory properties and shelf life of the product. In a recent experiment, eggs enriched with CLAs stored at 4 °C were hard-cooked and demonstrated very different sensory characteristics from those of control eggs not enriched with CLAs. The egg white of the CLA-enriched eggs (excessive enrichment) became discolored, and the egg yolk increased in size with storage (*17*). This phenomenon might reflect a compromise of the egg stability and sensory properties.

* To whom correspondence should be addressed: Bruce A. Watkins, Professor and University Faculty Scholar, Purdue University, Center for Enhancing Foods to Protect Health, 745 Agriculture Mall Drive, West Lafayette, IN 47907-2009. Telephone: 765-494-5802. Fax: 765-494-7953. E-mail: baw@purdue.edu.
† Purdue University.
‡ Colorado State University.

10.1021/jf026156v CCC: $25.00 © 2003 American Chemical Society
Published on Web 10/11/2003

No studies have been reported that examined this aspect of CLA-enriched eggs.

The objective of this investigation was to determine why higher incorporation of CLAs into eggs altered the physical and sensory properties of egg albumen and yolk in stored eggs. The current study utilized gas chromatography (GC) to analyze the fatty acid composition of eggs and to follow the distribution of CLAs in the egg components in the presence and absence of docosahexaenoic acid (DHA) enrichment of eggs. Dietary lipid treatments included both DHA and CLAs in the study. The information obtained from this research will be important for developing designer eggs that could be used to deliver the health benefits of CLAs and DHA and help fulfill the newly revised Dietary Reference Intakes (DRIs) for omega-3 fatty acids (www.nap.edu).

MATERIALS AND METHODS

Animals and Diets. Single Comb White Leghorn (SCWL) laying hens were randomly assigned to 40 cages (one hen per cage). The hens were divided into four treatment groups and administered different dietary lipid sources. Diets were formulated from a typical corn−soybean basal diet to contain all of the nutrients required by the laying hen (*18*). The treatments included one of four diets containing safflower oil (820 g in 19.2 kg of basal diet, SAFF, control group), DHA (500 g of DHA source + 540 g of safflower oil in 19.2 kg of basal diet), a combination of CLAs and DHA (500 g of DHA source + 540 g of CLA oil in 19.2 kg of basal diet), and CLAs alone (540 g of CLA oil + 280 g of safflower oil in 19.2 kg of basal diet). Diets were prepared every 2 weeks and kept at 4 °C until fed to the hens. The CLA supplement was an oil (PharmaNutrients, Lake Bluff, IL), and the DHA was a free-flowing powder [DHA GOLD, OmegaTech (now Martek Biosciences Boulder Corp.), Boulder, CO]. Upon analysis, the CLA oil contained 75% CLA isomers [35.07% 18:2(*c*9,*t*11), 35.53% 18:2(*t*10,*c*12), 1.46% 18:2(*c*,*c*), 2.07% 18:2(*t*,*t*), 4.97% 16:0, 15.33% 18:1, and 0.77% 18:2n-6], and the DHA source was 56% oil, of which 30.4% was DHA.

Sample Collections. Eggs were collected after 12 weeks of feeding and stored at 4 °C for subsequent analysis. The fatty acid methyl esters (FAMEs) prepared from lipids isolated from different egg compartments and from polar and neutral lipid fractions were characterized by GC.

Isolation of Yolk Granules and Plasma. A 5-g sample of yolk was weighed, and 5 mL of saline solution was added to the yolk to make a 1:1 dilution. The diluted sample was then vortexed to ensure even suspension of the granules. The mixture was centrifuged at 80 000*g* for 40 min (*19*), and both the supernatant (which contains the plasma) and the pellet (which contains the granules) were collected. Egg albumen was also collected. The three egg components were freeze-dried and stored in desiccators until analysis.

Isolation of Vitelline Membrane. Eggs were cracked and yolks removed into a sterile Petri dish where they were punctured with a Pasteur pipet. The yolk vitelline membrane was freed of yolk by being washed with distilled water until the washing liquid was clear. After excess water had been removed, the membrane was placed in 7 mL of methanol for lipid extraction.

Lipid Extraction and Separation of Polar and Neutral Lipids. An aliquot of each yolk plasma, egg white, and yolk granule was weighed, and lipid was extracted with chloroform/methanol (2:1, v/v) (*20*, *21*). In the case of vitelline membrane, one whole membrane was used for fatty acid analysis. Four eggs from each treatment group were analyzed in the study.

The total lipids from the egg yolk were further separated into polar and neutral lipids by solid-phase extraction (SPE). About 60 mg of lipids was dissolved into 0.5 mL of solvent and loaded onto a 600-mg silica cartridge (Alltech, Deerfield, IL) preconditioned with *tert*-butyl methyl ether (tBuOMe). The cartridge was treated with 5 mL of tBuOMe and the eluate contained the neutral lipids. After the cartridge had been washed with 10 mL of tBuOMe, the polar lipids were eluted with 20 mL of methanol. Both fractions were dried under a stream of N_2 gas at 25 °C and dissolved in solvent for further analysis (*22*).

Methylation of Lipid. The total lipids were methylated by sodium methoxide in methanol to maximize the characterization of the CLA isomeric distribution of the FAMEs (*12*). The FAME concentration was then adjusted for GC analysis. A saponification and acid-catalyzed methylation method was used to derivatize the CLA isomers in polar lipids (*23*, *24*). A volume corresponding to 10 mg of polar lipids was placed in a 15-mL Teflon-lined screw-cap tube. The lipid sample was dried under a stream of N_2 gas at 30 °C, 0.2 mL of NaOH−MeOH (0.5 N) added to the lipid, and the sample vortex-mixed and heated at 100 °C for 5 min. The sample was cooled in cold water, 0.5 mL of HCl−MeOH (4%) added to the sample, and the sample vortex-mixed and held at room temperature for 5 min. Approximately 5 mL of isooctane and 3 mL of distilled water were added to extract the FAMEs. The mixture was shaken for 10 min and centrifuged at 3000 rpm for 5 min, and then the upper phase was collected, dried with sodium sulfate, and concentrated with nitrogen gas for GC analysis. The neutral lipids were methylated in a similar manner except that saponification was carried out at 50 °C.

GC Analysis. A gas chromatograph (Agilent 6890 Series) equipped with an autosampler (model 7683), GC ChemStation (rev. A.08.03), and flame ionization detector (FID) (Agilent Technologies, Inc., Wilmington, DE) was used to analyze the FAMEs. The GC was operated at a temperature of 140 °C for 2 min, followed by heating at 1.5 °C/min to 198 °C, and holding for 7 min. A DB-23 column was used for the analysis (30 m, 0.53 mm i.d., 0.5 μm film thickness; Agilent Technologies, Inc., Wilmington, DE). The injector and FID were maintained at 225 and 250 °C, respectively. Identification of sample FAMEs was achieved by comparing the retention times to authentic FAME standards (Matreya Inc., Pleasant Cap, PA, and Nu-Chek-Prep, Elysian, MN).

Fat Content Determination. A 0.5-g sample of the dried plasma or egg albumen was weighed, and the lipid extracted with chloroform/methanol (2:1, v/v). The solvent in the extracted lipid was dried, and the lipid sample weighed.

Statistical Analysis. The data collected were analyzed by a one-way analysis of variance (ANOVA). Differences were further analyzed with the Student−Newman−Keuls (SNK) test or Tukey's range test, with $p < 0.05$ indicating a significant difference (SAS software package for UNIX; SAS Institute Inc., Cary, NC). Variations between treatment groups are expressed as the pooled standard error of the mean (SEM) or mean ± SEM, where applicable.

RESULTS AND DISSCUSSION

Integration of CLAs into the Egg and Fatty Acid Compositional Changes. To explain how CLAs altered the physical properties of the egg, analyses were performed to determine whether CLAs are preferentially targeted to specific compartments. The GC analysis of FAMEs included egg yolk, egg albumen, vitelline membrane, yolk plasma, and yolk granules. The results showed that feeding hens CLAs for 10 weeks led to a significant enrichment of CLA isomers in egg yolk (treatments CAD and CLAs), independent of DHA supplementation (**Tables 1** and **2**). The supplementation of DHA to hens elevated the amount of this fatty acid in egg yolk. Moreover, the combination of CLAs and DHA (treatment CAD) appeared to synergistically elevate DHA in yolk lipids (**Tables 1** and **2**).

The FAME analysis revealed that, in both neutral and polar lipid classes and in all egg components examined, supplementing hens with CLAs caused a significant enrichment of all isomers compared to egg samples from the SAFF group. Both CLA and DHA supplements resulted in increased concentrations of these fatty acids in lipid classes of egg albumen, vitelline membrane, and yolk plasma and granules. The treatments containing DHA also resulted in a significantly lower ratio of n-6 to n-3 fatty acids in yolk neutral and polar lipids of egg compartments. The combination treatment of CAD resulted in distinctive changes in the content of some long-chain polyunsaturated fatty acids (PUFAs). CAD treatment greatly elevated the levels of 22:5n-3

Table 1. Fatty Acid Composition (g/100 g) of Neutral Lipid from Egg Yolk[a]

fatty acid	dietary treatment[b] SAFF	DHA	CAD	CLA	pooled SEM	ANOVA *p* value
16:0	24.45^{B}	24.99^{B}	29.20^{A}	28.97^{A}	0.41	0.0001
16:1n-7	1.61^{A}	1.81^{A}	0.50^{B}	0.44^{B}	0.07	0.0001
17:0	0.21^{B}	0.20^{B}	0.36^{A}	0.31^{A}	0.02	0.0003
18:0	9.17^{C}	8.12^{D}	15.84^{A}	14.78^{B}	0.31	0.0001
18:1n-9	31.34^{A}	31.58^{A}	16.62^{B}	16.56^{B}	1.02	0.0001
18:2n-6	28.02^{A}	26.84^{A}	18.45^{C}	24.54^{B}	0.63	0.0001
18:3n-6	0.16^{A}	0.05^{B}	ND^{B}	ND^{B}	0.03	0.004
18:3n-3	0.49^{B}	0.66^{AB}	0.85^{A}	0.76^{A}	0.06	0.005
18:2(*c*9,*t*11)	ND^{B}	ND^{B}	7.42^{A}	6.72^{A}	0.27	0.0001
18:2(*t*10,*c*12)	ND^{B}	ND^{B}	2.72^{A}	2.70^{A}	0.20	0.0001
18:2(*c*,*c*)	ND^{C}	ND^{C}	0.40^{B}	0.50^{A}	0.03	0.0001
18:2(*t*,*t*)	ND	ND	0.08	0.13	0.03	NS
20:1n-9	0.14	0.12	ND	ND	0.05	NS
20:2n-6	0.27^{A}	0.21^{A}	ND^{B}	0.18^{A}	0.03	0.0004
20:3n-6	0.16^{A}	0.19^{A}	ND^{B}	ND^{B}	0.04	0.01
20:4n-6	1.49^{A}	1.35^{A}	0.78^{B}	0.96^{B}	0.06	0.0001
22:5n-6	0.17^{B}	0.04^{C}	0.40^{A}	ND^{C}	0.04	0.0001
22:5n-3	ND^{B}	ND^{B}	0.22^{A}	ND^{B}	0.01	0.0001
22:6n-3	0.33^{C}	1.73^{B}	3.98^{A}	0.15^{C}	0.15	0.0001
SAT	33.83^{C}	33.31^{C}	45.40^{A}	44.06^{B}	0.35	0.0001
MONO	33.08^{A}	33.51^{A}	17.12^{B}	17.01^{B}	1.09	0.0001
PUFAs	31.09^{A}	31.08^{A}	24.68^{B}	26.59^{B}	0.72	0.0001
n-6	30.27^{A}	28.69^{A}	19.63^{C}	25.68^{B}	0.71	0.0001
n-3	0.82^{C}	2.39^{B}	5.05^{A}	0.91^{C}	0.15	0.0001
n-6/n-3	36.80^{A}	12.15^{C}	3.92^{D}	29.19^{B}	1.58	0.0001
SAT/PUFAs	1.09^{C}	1.07^{C}	1.84^{A}	1.66^{B}	0.03	0.0001
total CLA	ND^{B}	ND^{B}	10.62^{A}	10.05^{A}	0.49	0.0001

[a] Means ($n = 4$) within a row having different superscripts are significantly different by one-way ANOVA followed by Student–Newman–Keuls (SNK) test ($p < 0.05$). [b] SAFF, safflower oil treatment; CLA, conjugated linoleic acid treatment; DHA, docosahexaenoic acid treatment; CAD, combination of CLA and DHA treatment; ND, not detected; NS, not significant; SAT, total saturated fatty acids; MONO, total monounsaturated fatty acids; PUFAs, total polyunsaturated fatty acids; n-6, total omega-6 fatty acids; n-3, total omega-3 fatty acids; n-6/n-3, ratio of omega-6 to omega-3 fatty acids; SAT/PUFAs, ratio of saturated to polyunsaturated fatty acids.

Table 2. Fatty Acid Composition (g/100 g) of Polar Lipid from Egg Yolk[a]

fatty acid	dietary treatment[b] SAFF	DHA	CAD	CLA	pooled SEM	ANOVA *p* value
16:0	28.10^{B}	29.36^{AB}	31.20^{A}	25.94^{C}	0.62	0.0005
16:1n-7	0.35^{A}	0.53^{A}	ND^{B}	0.10^{B}	0.07	0.0004
17:0	0.14^{B}	0.20^{AB}	0.27^{A}	0.26^{A}	0.03	0.03
18:0	16.41^{B}	15.33^{B}	14.43^{B}	19.83^{A}	0.68	0.0006
18:1n-9	18.62^{A}	18.80^{A}	11.70^{B}	12.48^{B}	0.43	0.0001
18:2n-6	23.36^{A}	19.65^{B}	12.45^{C}	22.62^{A}	0.43	0.0001
18:3n-3	ND	ND	0.17	0.04	0.06	NS
18:2(*c*9,*t*11)	ND^{C}	ND^{C}	5.71^{B}	6.87^{A}	0.33	0.0001
18:2(*t*10,*c*12)	ND^{B}	ND^{B}	2.82^{A}	3.06^{A}	0.17	0.0001
18:2(*c*,*c*)	ND^{C}	ND^{C}	0.42^{B}	0.65^{A}	0.04	0.0001
18:2(*t*,*t*)	ND^{B}	ND^{B}	ND^{B}	0.12^{A}	0.02	0.002
20:1n-9	ND	ND	0.08	ND	0.04	NS
20:2n-6	0.40^{A}	0.28^{A}	ND^{B}	0.27^{A}	0.05	0.0006
20:3n-6	0.45^{B}	0.54^{A}	0.25^{D}	0.32^{C}	0.02	0.0001
20:4n-6	7.52^{A}	5.60^{B}	3.04^{D}	4.17^{C}	0.23	0.0001
20:5n-3	ND^{B}	0.04^{B}	0.24^{A}	ND^{B}	0.02	0.0001
22:4n-6	0.35^{A}	0.11^{B}	ND^{C}	0.21^{B}	0.03	0.0001
22:5n-6	1.22^{B}	0.76^{C}	1.53^{A}	0.50^{D}	0.07	0.0001
22:5n-3	ND^{C}	ND^{C}	0.36^{A}	0.20^{B}	0.01	0.0001
22:6n-3	1.91^{C}	7.24^{B}	14.47^{A}	0.90^{C}	0.50	0.0001
SAT	44.66	44.89	45.89	46.03	0.36	NS
MONO	18.97^{A}	19.33^{A}	11.78^{B}	12.58^{B}	0.45	0.0001
PUFAs	35.21^{A}	34.21^{B}	32.50^{C}	29.24^{D}	0.28	0.0001
n-6	33.30^{A}	26.93^{C}	17.26^{D}	28.09^{B}	0.37	0.0001
n-3	1.91^{C}	7.28^{B}	15.23^{A}	1.15^{C}	0.51	0.0001
n-6/n-3	17.43^{B}	3.92^{C}	1.14^{D}	24.52^{A}	0.47	0.0001
SAT/PUFAs	1.27^{C}	1.31^{C}	1.41^{B}	1.57^{A}	0.02	0.0001
total CLA	ND^{C}	ND^{C}	8.95^{B}	10.70^{A}	0.54	0.0001

[a] Means ($n = 4$) within a row having different superscripts are significantly different by one-way ANOVA followed by Student–Newman–Keuls (SNK) test ($p < 0.05$). [b] SAFF, safflower oil treatment; CLA, conjugated linoleic acid treatment; DHA, docosahexaenoic acid treatment; CAD, combination of CLA and DHA treatment; ND, not detected; NS, not significant; SAT, total saturated fatty acids; MONO, total monounsaturated fatty acids; PUFAs, total polyunsaturated fatty acids; n-6, total omega-6 fatty acids; n-3, total omega-3 fatty acids; n-6/n-3, ratio of omega-6 to omega-3 fatty acids; SAT/PUFAs, ratio of saturated to polyunsaturated fatty acids.

and 22:6n-3 and decreased the amount of 20:4n-6 compared to treatments that contained either DHA or CLAs, showing a clear preserving effect of CLAs on n-3 PUFA content and an inhibitory effect on n-6 PUFAs in the egg.

Distribution of CLAs in Neutral and Polar Lipids. Molecular polarity influences the physical properties of compounds and their distribution in biological systems. To determine how CLAs partitioned into different lipid fractions, the yolk lipids were separated into neutral (N) and polar (P) classes. Upon GC analysis of the FAMEs, the percentages of various CLA isomers were nearly identical in the two lipid fractions (**Tables 1** and **2**). In egg yolk N lipid, CLA supplementation resulted in higher concentrations of 16:0, 18:0, and total saturated fatty acids, and the ratio of total saturated fatty acids (SAT) to PUFAs. The amounts of 16:1n-7, 18:1n-9, monounsaturated fatty acids (MONO), PUFAs, and total n-6 were decreased (**Table 1**). Although the DHA supplementation did result in an increase of this fatty acid in yolk N lipid, the DHA treatment did not significantly modify the fatty acid composition of N lipid. In egg yolk P lipid, the amount of 18:0 and the ratios of n-6 to n-3 fatty acids and SAT to PUFAs were increased, whereas the amounts of 18:1n-9, 20:4n-6, 22:5n-6, 22:6n-3, MONO, PUFAs, and total n-6 were decreased with the CLA treatments (**Table 2**). All isomers of CLAs were detected in both N and P lipids, and the total amounts of CLAs were similar. The total amount of DHA was greater in P than in N lipids, and an interesting finding was that supplementation with both DHA and CLAs (treatment CAD) resulted in a surprisingly high concentration of 22:6n-3 in P lipid. The P lipid fraction from egg yolks of hens supplemented with CAD appears to reflect a synergistic effect on DHA accumulation that was associated with lower contents of 20:4n-6 and total n-6 PUFAs. CLAs were detected in both N and P lipids; however, because there is more N lipid in the yolk than P lipid, the total amount of CLAs in N lipid of yolk lipoproteins accreted by the hen represents a significantly higher amount of these isomers in yolk.

The polarity of a molecule will determine its hydrophobicity/hydrophilicity. Incorporation of large amounts of CLAs into the egg yolk can potentially change the molecular interactions among components inside the yolk, that is, the structure and hydrophilicity could be altered. If the hydrophilicity of the yolk is increased, the osmolarity between the yolk and the albumen will be disrupted, and water from the egg albumen will move into the yolk compartment (*25*). Increased water content might change the physical properties of the yolk. In addition, as more water moves into the yolk, the volume of the yolk will increase; this will inflate the vitelline membrane so that the changes in permeability will allow it to accommodate larger molecules. The larger size of egg yolks obtained from hens given the CLA supplements in this study would explain the proposed osmotic effect of CLA isomers.

In egg albumen, the contents of 16:0, 18:0, 18:2n-6, 18:3n-3, and SAT and the ratio of n-6 to n-3 fatty acids were increased, whereas the contents of 20:4n-6, 22:5n-6, and total

Table 3. Fatty Acid Composition (g/100 g) of Egg Albumen[a]

fatty acid	dietary treatment[b] SAFF	DHA	CAD	CLA	pooled SEM	ANOVA *p* value
16:0	18.30^{B}	17.05^{B}	28.54^{A}	27.10^{A}	1.02	0.0001
16:1n-7	0.81	0.62	0.50	0.48	0.13	NS
17:0	0.27^{AB}	0.18^{B}	0.34^{A}	0.30^{AB}	0.03	0.04
18:0	7.76^{B}	6.09^{B}	17.26^{A}	17.13^{A}	0.89	0.0001
18:1n-9	16.66	17.05	15.02	17.82	2.54	NS
18:2n-6	20.92^{A}	15.41^{B}	15.52^{B}	23.66^{A}	1.40	0.003
18:3n-6	0.23	0.19	ND	ND	0.07	NS
18:3n-3	0.15^{C}	ND^{C}	0.66^{A}	0.49^{B}	0.05	0.0001
18:2(*c*9,*t*11)	ND^{C}	ND^{C}	6.27^{A}	4.82^{B}	0.32	0.0001
18:2(*t*10,*c*12)	ND^{C}	ND^{C}	2.39^{A}	1.80^{B}	0.15	0.0001
18:2(*c*,*c*)	ND^{B}	ND^{B}	0.31^{A}	0.43^{A}	0.05	0.0001
18:2(*t*,*t*)	ND^{B}	ND^{B}	0.60^{A}	0.42^{A}	0.06	0.0001
20:1n-9	0.06^{B}	0.41^{A}	ND^{B}	ND^{B}	0.05	0.0001
20:2n-6	0.23^{B}	0.58^{A}	ND^{B}	0.19^{B}	0.08	0.002
20:3n-6	0.40^{A}	0.46^{A}	ND^{B}	ND^{B}	0.03	0.0001
20:4n-6	9.92^{A}	7.77^{A}	1.13^{B}	1.30^{B}	1.05	0.0001
20:5n-3	ND^{B}	0.50^{A}	ND^{B}	ND^{B}	0.05	0.0001
22:4n-6	1.14^{A}	0.73^{A}	ND^{B}	0.08^{B}	0.13	0.0001
22:5n-6	0.90^{A}	1.10^{A}	0.83^{A}	0.27^{B}	0.12	0.002
22:5n-3	0.37^{A}	0.56^{A}	0.38^{A}	ND^{B}	0.08	0.003
22:6n-3	2.07^{C}	10.56^{A}	7.41^{B}	0.48^{C}	0.80	0.0001
SAT	26.34^{B}	23.32^{B}	46.15^{A}	44.52^{A}	1.79	0.0001
MONO	17.53	18.08	15.51	18.31	2.54	NS
PUFAs	36.33^{A}	37.87^{A}	25.93^{B}	26.47^{B}	1.46	0.0001
n-6	33.74^{A}	26.25^{B}	17.48^{C}	25.51^{B}	1.22	0.0001
n-3	2.59^{C}	11.62^{A}	8.45^{B}	0.96^{C}	0.84	0.0001
n-6/n-3	13.84^{B}	2.38^{B}	2.12^{B}	30.00^{A}	3.33	0.0002
SAT/PUFAs	0.73^{B}	0.62^{B}	1.79^{A}	1.70^{A}	0.10	0.0001
total CLA	ND^{C}	ND^{C}	9.57^{A}	7.48^{B}	0.48	0.0001

[a] Means ($n = 4$) within a row having different superscripts are significantly different by one-way ANOVA followed by Student–Newman–Keuls (SNK) test ($p < 0.05$). [b] SAFF, safflower oil treatment; CLA, conjugated linoleic acid treatment; DHA, docosahexaenoic acid treatment; CAD, combination of CLA and DHA treatment; ND, not detected; NS, not significant; SAT, total saturated fatty acids; MONO, total monounsaturated fatty acids; PUFAs, total polyunsaturated fatty acids; n-6, total omega-6 fatty acids; n-3, total omega-3 fatty acids; n-6/n-3, ratio of omega-6 to omega-3 fatty acids; SAT/PUFAs, ratio of saturated to polyunsaturated fatty acids.

PUFAs and n-6 were decreased by CLA supplementation (**Table 3**). The supplementation of DHA also resulted in an increase of this fatty acid in egg albumen. The increase of DHA in egg albumen occurred independently of CLA supplementation, as did the decrease in the ratio of n-6 to n-3 fatty acids.

CLAs Might Damage the Integrity of the Vitelline Membrane. CLA supplementation (treatments CAD and CLA) resulted in higher amounts of 18:0 and SAT and higher ratios of SAT to PUFAs but decreased amounts of 16:1n-7, 18:1n-9, and MONO in the vitelline membrane compared to the control (**Table 4**). In liver, cyclopropene fatty acids have been reported to induce changes in eggs by reacting with hydroxyl groups of the fatty acid desaturase enzyme system (*26*−*28*). In our study, the fact that eggs from hens administered CLAs (treatments CAD and CLA) exhibited a higher amount of saturated fatty acids than found in the control eggs is highly suggestive that CLAs might exhibit behavior similar to that reported previously for cyclopropene fatty acids in the hen (*29*, *30*). Moreover, mottling of the egg yolk reported to occur with treatment of cyclopropene fatty acids (*31*) was also observed in eggs enriched with the high amount of CLA isomers supplemented in the present study. The albumen was discolored, and the size of the egg yolk increased as the duration of feeding of the hens increased. This phenomenon was consistent in eggs containing CLAs and those containing cyclopropene fatty acids. It is therefore reasonable to assume that CLAs might alter fatty acid synthesis in the liver of the hen as do cyclopropene fatty acids. This notion is further supported by a recently published report demonstrating that CLAs decrease hepatic stearoyl−CoA desaturase mRNA expression (*32*), which is the very enzyme with which cyclopropene reacts.

Table 4. Fatty Acid Composition (g/100 g) of Vitelline Membrane[a]

fatty acid	dietary treatment[b] SAFF	DHA	CAD	CLA	pooled SEM	ANOVA *p* value
16:0	23.23	23.14	27.56	26.92	1.14	NS
16:1n-7	1.15^{A}	1.44^{A}	0.46^{B}	0.44^{B}	0.11	0.0001
17:0	0.30^{B}	0.27^{B}	0.37^{A}	0.36^{A}	0.01	0.001
18:0	11.66^{B}	8.74^{C}	17.21^{A}	17.23^{A}	0.78	0.0001
18:1n-9	27.02^{A}	26.95^{A}	13.72^{B}	16.67^{B}	1.28	0.0001
18:2n-6	22.00	19.79	13.72	21.36	1.54	0.01
18:3n-6	0.05	ND	ND	ND	0.02	NS
18:3n-3	0.26^{C}	0.42^{BC}	0.65^{A}	0.49^{AB}	0.05	0.002
18:2(*c*9,*t*11)	0.09^{B}	ND^{B}	5.67^{A}	4.84^{A}	0.42	0.0001
18:2(*t*10,*c*12)	ND^{C}	ND^{C}	2.35^{A}	1.76^{B}	0.17	0.0001
18:2(*c*,*c*)	ND^{B}	ND^{B}	0.20^{AB}	0.37^{A}	0.06	0.002
18:2(*t*,*t*)	ND	ND	0.10	ND	0.05	NS
20:2n-6	0.13	ND	ND	ND	0.04	NS
20:3n-6	0.15	0.16	ND	ND	0.06	NS
20:4n-6	2.36^{A}	1.62^{B}	0.84^{C}	1.47^{B}	0.17	0.0003
22:4n-6	0.17	ND	ND	ND	0.05	NS
22:5n-6	0.68^{A}	0.34^{B}	0.80^{A}	0.08^{C}	0.07	0.0001
22:6n-3	1.00^{C}	2.95^{B}	5.73^{A}	0.26^{D}	0.24	0.0001
SAT	35.18^{B}	32.15^{B}	45.14^{A}	44.51^{A}	1.63	0.0002
MONO	28.17	28.39	14.18	17.11	1.37	0.0001
PUFAs	26.80	25.27	21.74	23.66	1.77	NS
n-6	25.54^{A}	21.91^{A}	15.36^{B}	22.91^{A}	1.63	0.006
n-3	1.26^{C}	3.36^{B}	6.38^{A}	0.75^{C}	0.26	0.0001
n-6/n-3	25.36^{A}	6.58^{B}	2.41^{B}	31.57^{A}	4.01	0.0005
SAT/PUFAs	1.34^{B}	1.29^{B}	2.07^{A}	1.91^{A}	0.11	0.0005
total CLA	0.09^{B}	ND^{B}	8.32^{A}	6.97^{A}	0.62	0.0001

[a] Means ($n = 4$) within a row having different superscripts are significantly different by one-way ANOVA followed by Student–Newman–Keuls (SNK) test ($p < 0.05$). [b] SAFF, safflower oil treatment; CLA, conjugated linoleic acid treatment; DHA, docosahexaenoic acid treatment; CAD, combination of CLA and DHA treatment; ND, not detected; NS, not significant; SAT, total saturated fatty acids; MONO, total monounsaturated fatty acids; PUFAs, total polyunsaturated fatty acids; n-6, total omega-6 fatty acids; n-3, total omega-3 fatty acids; n-6/n-3, ratio of omega-6 to omega-3 fatty acids; SAT/PUFAs, ratio of saturated to polyunsaturated fatty acids.

CLAs in Yolk Plasma and Granules. The CLA isomers were incorporated into both yolk plasma and granules (**Tables 5** and **6**). In yolk plasma, the contents of 16:0, 17:0, 18:0, and SAT and the ratio of SAT to PUFAs were increased, whereas the contents of 16:n-7, 18:1n-9, 22:5n-6, MONO, PUFAs, and n-6 were decreased with CLA treatment. In yolk granules, the amounts of 16:0, 18:0, and SAT and the ratios of n-6 to n-3 fatty acids and SAT to PUFAs were increased, whereas the amounts of 16:1n-7, 18:1n-9, 18:2n-6, 20:4n-6, 22:5n-6, MONO, PUFAs, and total n-6 were decreased with CLA supplementation of hens. In both yolk plasma and granules, DHA was increased as a result of supplementation. In addition, CLA supplementation resulted in a decrease of 20:4n-6 in these yolk fractions compared to samples obtained from the control (treatment SAFF).

Because there were more lipids in the plasma, it can be concluded that a greater amount of the CLAs was incorporated into the yolk plasma. The fatty acid profiles of both plasma and granule reflect the fatty acid composition of the egg yolk analysis (**Tables 1** and **2**). However, because the plasma is a true solution whereas the granules represent yolk solids, the CLAs in the two fractions could be different in terms of their contributions to the change in physical properties of the yolk.

CLAs resulted in major fatty acid compositional changes of

Table 5. Fatty Acid Composition (g/100 g) of Egg Yolk Plasma[a]

fatty acid	dietary treatment[b] SAFF	DHA	CAD	CLA	pooled SEM	ANOVA *p* value
16:0	22.49[D]	24.40[C]	29.62[A]	27.74[B]	0.42	0.0001
16:1n-7	1.32[A]	1.69[A]	0.46[B]	0.44[B]	0.15	0.0001
17:0	0.22[B]	0.20[B]	0.36[A]	0.34[A]	0.01	0.0001
18:0	11.22[B]	9.14[B]	20.04[A]	19.02[A]	0.89	0.0001
18:1n-9	31.28[B]	34.62[A]	15.52[D]	18.71[C]	0.71	0.0001
18:2n-6	27.60[A]	22.92[B]	14.77[C]	22.74[B]	0.95	0.0001
18:3n-6	0.18[A]	0.09[B]	ND[B]	ND[B]	0.03	0.0009
18:3n-3	0.43[B]	0.53[B]	0.65[A]	0.50[B]	0.03	0.006
18:2(*c*9,*t*11)	ND[C]	ND[C]	6.49[A]	5.29[B]	0.29	0.0001
18:2(*t*10,*c*12)	ND[C]	ND[C]	2.71[A]	1.87[B]	0.10	0.0001
18:2(*c*,*c*)	ND[B]	ND[B]	0.39[A]	0.39[A]	0.01	0.0001
18:2(*t*,*t*)	ND[B]	ND[B]	0.20[A]	0.08[B]	0.02	0.0001
20:1n-9	0.18[A]	0.05[B]	ND[B]	ND[B]	0.03	0.002
20:2n-6	0.31[A]	0.17[A]	ND[B]	0.18[A]	0.04	0.003
20:3n-6	0.27[A]	0.20[A]	ND[B]	ND[B]	0.04	0.0004
20:4n-6	2.36[A]	1.42[B]	0.79[C]	1.33[B]	0.08	0.0001
22:4n-6	0.16[A]	ND[B]	ND[B]	ND[B]	0.03	0.003
22:5n-6	0.55[A]	0.29[B]	0.65[A]	0.05[C]	0.05	0.0001
22:5n-3	ND[B]	ND[B]	0.35[A]	ND[B]	0.01	0.0001
22:6n-3	0.60[C]	3.11[B]	5.79[A]	0.27[C]	0.13	0.0001
SAT	33.92[B]	33.75[B]	50.01[A]	47.10[A]	0.96	0.0001
MONO	32.78[B]	36.36[A]	15.98[D]	19.14[C]	0.83	0.0001
PUFAs	32.48[A]	28.72[B]	23.00[C]	25.08[C]	1.05	0.0001
n-6	31.44[A]	25.08[B]	16.22[C]	24.31[B]	0.99	0.0001
n-3	1.03[C]	3.63[B]	6.78[A]	0.77[C]	0.14	0.0001
n-6/n-3	30.49[A]	6.98[B]	2.39[C]	31.76[A]	0.83	0.0001
SAT/PUFAs	1.05[B]	1.18[B]	2.18[A]	1.92[A]	0.12	0.0001
total CLA	ND[C]	ND[C]	9.79[A]	7.62[B]	0.40	0.0001

[a] Means ($n = 4$) within a row having different superscripts are significantly different by one-way ANOVA followed by Student–Newman–Keuls (SNK) test ($p < 0.05$). [b] SAFF, safflower oil treatment; CLA, conjugated linoleic acid treatment; DHA, docosahexaenoic acid treatment; CAD, combination of CLA and DHA treatment; ND, not detected; SAT, total saturated fatty acids; MONO, total monounsaturated fatty acids; PUFAs, total polyunsaturated fatty acids; n-6, total omega-6 fatty acids; n-3, total omega-3 fatty acids; n-6/n-3, ratio of omega-6 to omega-3 fatty acids; SAT/PUFAs, ratio of saturated to polyunsaturated fatty acids.

Table 6. Fatty Acid Composition (g/100 g) of the Egg Yolk Granules[a]

fatty acid	dietary treatment[b] SAFF	DHA	CAD	CLA	pooled SEM	ANOVA *p* value
16:0	24.24[D]	26.57[C]	30.91[A]	28.39[B]	0.38	0.0001
16:1n-7	1.18[B]	1.61[A]	0.44[C]	0.40[C]	0.11	0.0001
17:0	0.16[B]	0.15[B]	0.33[A]	0.32[A]	0.04	0.008
18:0	12.40[B]	9.80[C]	18.88[A]	19.09[A]	0.73	0.0001
18:1n-9	28.48[B]	31.13[A]	14.87[D]	17.76[C]	0.78	0.0001
18:2n-6	26.81[A]	21.94[C]	14.50[C]	22.88[B]	0.80	0.0001
18:3n-3	0.26[B]	0.43[AB]	0.58[A]	0.43[AB]	0.05	0.007
18:2(*c*9,*t*11)	ND[C]	ND[C]	6.21[A]	5.07[B]	0.26	0.0001
18:2(*t*10,*c*12)	ND[C]	ND[C]	2.69[A]	1.88[B]	0.10	0.0001
18:2(*c*,*c*)	ND[B]	ND[B]	0.30[A]	0.42[A]	0.05	0.0001
18:2(*t*,*t*)	ND	ND	0.10	ND	0.05	NS
20:2n-6	0.23[A]	ND[B]	ND[B]	ND[B]	0.04	0.003
20:3n-6	0.27	0.25	ND	ND	0.07	NS
20:4n-6	3.53[A]	2.14[B]	1.08[C]	2.03[B]	0.13	0.0001
22:5n-6	0.83[A]	0.43[B]	0.81[A]	0.08[C]	0.08	0.0001
22:5n-3	ND[B]	ND[B]	0.28[A]	ND[B]	0.05	0.003
22:6n-3	0.93[C]	4.57[B]	7.38[A]	0.40[C]	0.21	0.0001
SAT	36.80[C]	36.53[C]	50.12[A]	47.79[B]	0.66	0.0001
MONO	29.66[B]	32.74[A]	15.31[D]	18.16[C]	0.86	0.0001
PUFAs	32.86[A]	29.76[B]	24.63[C]	25.82[C]	0.89	0.0001
n-6	31.67[A]	24.76[B]	16.39[C]	24.99[B]	0.77	0.0001
n-3	1.19[C]	5.00[B]	8.24[A]	0.83[C]	0.22	0.0001
n-6/n-3	26.67[B]	5.03[C]	1.99[D]	30.41[A]	0.64	0.0001
SAT/PUFAs	1.12[B]	1.23[B]	2.04[A]	1.87[A]	0.08	0.0001
total CLA	ND[C]	ND[C]	9.30[A]	7.38[B]	0.39	0.0001

[a] Means ($n = 4$) within a row having different superscripts are significantly different by one-way ANOVA followed by Student–Newman–Keuls (SNK) test ($p < 0.05$). [b] SAFF, safflower oil treatment; CLA, conjugated linoleic acid treatment; DHA, docosahexaenoic acid treatment; CAD, combination of CLA and DHA treatment; ND, not detected; NS, not significant; SAT, total saturated fatty acids; MONO, total monounsaturated fatty acids; PUFAs, total polyunsaturated fatty acids; n-6, total omega-6 fatty acids; n-3, total omega-3 fatty acids; n-6/n-3, ratio of omega-6 to omega-3 fatty acids; SAT/PUFAs, ratio of saturated to polyunsaturated fatty acids.

Table 7. Lipid Content (g/100 g) of Dried Yolk Plasma and Egg Albumen[a]

sample	dietary treatment[b] SAFF	DHA	CAD	CLA	ANOVA *p* value
plasma	0.68 ± 0.023[AB]	0.69 ± 0.004[A]	0.59 ± 0.013[C]	0.59 ± 0.029[BC]	0.007
albumen	0.0005 ± 0.0003	0.0006 ± 0.0003	0.0045 ± 0.001	0.0142 ± 0.008	NS

[a] Means ($n = 4$) within a row having different superscripts are significantly different by one-way ANOVA followed by Tukey's test ($p < 0.05$). [b] SAFF, safflower oil treatment; CLA, conjugated linoleic acid treatment; DHA, docosahexaenoic acid treatment; CAD, combination of CLA and DHA treatment; NS, not significant.

the eggs. It was demonstrated that CLAs caused significant changes in the fatty acid compositions in several components of eggs obtained from hens given dietary lipid supplements. Although some changes vary in distinct parts of the egg, a consistent robust finding was that lipid treatments increased the amounts of 16:0, 18:0, and SAT and the ratio of n-6 to n-3 fatty acids when hens were supplemented with CLAs. It is possible that CLAs inhibit the elongation and desaturation of 16:0 and 18:0 in the liver and result in the accumulation of saturated fatty acids, which leads to a compositional change in the fatty acids of egg yolk lipoproteins accreted by the hen. The fact that the fatty acid compositions of egg albumen and vitelline membrane mirrored that of the yolk might indicate that material exchange between egg yolk and albumen occurs. In addition, the amounts of MONO and PUFAs were decreased in eggs with CLA supplementation. Generally, CLAs reached a level of 10% of total fatty acids when incorporated into the yolk lipids.

Most of the yolk proteins and fatty acids are formed in the liver and transported into the yolk. Because CLAs accumulate in liver tissue of rats and other animals (*12*, *16*), the various isomers can directly influence fatty acid synthesis in the liver (*28*, *32*), which will ultimately alter the composition of the egg yolk. The dramatic change in quantity of fatty acids having a different polarity and hydrophobicity could be partially responsible for the change in physical properties of the egg.

Fat Content of Yolk Plasma and Egg White. To determine whether CLAs have any effects on the osmotic exchange between the egg yolk and the egg albumen, the fat contents of egg albumen and yolk plasma were determined. It was found that the yolk plasma obtained from the eggs of hens administered the CAD and CLA treatments contained significantly less lipid than found in the control eggs. The egg albumens of CLA- and CAD-treated groups showed a trend of greater lipid content than the control (**Table 7**). As expected, the egg yolk plasma contained more lipid than the egg albumen. These data were corroborated by GC analysis.

Vitelline membrane is the membrane that wraps the egg yolk. It forms a barrier between the yolk and the albumen. Permeability changes in this membrane could result in more intensive material exchange between the yolk and the albumen during storage. In the fat determination, it was found that the decrease in fat content in the yolk was significant at about 10%. A compositional change of this extent might impact the physical properties of the egg. Although the increase in fat content of the albumen was not as significant as the decrease in the yolk,

a considerable amount of fat could be defusing out of the yolk to the albumen. This is conceivable when comparing the sizes of the yolk and the total albumen. CLAs probably increase the permeability of the vitelline membrane and result in an increase in water migration from the albumen to the egg yolk (*25*, *33*). Such a change could facilitate lipid migration from the yolk to the albumen. The significant decrease in the lipid content could be partly responsible for the changes in physical properties of the egg.

Feeding CLAs to hens produced a rubbery egg yolk in hard-cooked eggs (*17*). This phenomenon is important because, when CLAs are incorporated into commercial eggs to achieve a health benefit, the sensory and quality properties of the food should not be compromised. Therefore, research is needed to determine the optimal levels of enrichment and elucidate how CLAs impact egg quality, and similarly for other nutraceutical fatty acids such as DHA. CLAs might affect several properties of egg lipids because they are incorporated into the egg structural polar lipids. According to our findings, CLAs might impart a more polar nature to the egg yolk lipid and thereby increase the permeability of the vitelline membrane.

Chemically, CLA isomers have unique double-bond structures that vary with respect to the position and geometry of the double-bond system. The conjugated double bonds could provide unique structural properties and reactivity to these isomers. Cyclopropene fatty acids have been suggested to react with sulfhydryl groups in the vitelline membrane (*33*). How CLAs might modify the permeability of the vitelline membrane remains to be determined.

In summary, the incorporation of CLAs into the egg altered its sensory properties. This change might be the result of the unique chemistry of the conjugated double bonds in CLA isomers. The modification of the fatty acid composition in the vitelline membrane might cause structural variations and modify the membrane permeability, which could facilitate and/or accelerate material exchange between the egg compartments. Gross compositional changes in the yolk could be responsible for the changes in physical properties, as well. Further investigation is needed to understand the interactions among the yolk components to determine the precise role that CLA isomers play in changing the functionality of the egg yolk.

ABBREVIATIONS USED

CLAs, conjugated linoleic acids; DHA, docosahexaenoic acid; PUFAs, polyunsaturated fatty acids; n-3, omega-3 fatty acids; n-6, omega-6 fatty acids.

ACKNOWLEDGMENT

The authors thank PharmaNutrients (Lake Bluff, IL) for donating the CLA oil and Martek Biosciences Boulder Corp. (Boulder, CO) for supplying the DHA supplement.

LITERATURE CITED

(1) Ip, C.; Banni, S.; Angioni, E.; Carta, G.; McGinley, J.; Thompson, H. J.; Barbano; D.; Bauman, D. Conjugated linoleic acid—enriched butter fat alters mammary gland morphogenesis and reduces cancer risk in rats. *J. Nutr.* **1999**, *129*, 2135−2142.

(2) Lee, K. N.; Kritchevsky, D.; Pariza, M. W. Conjugated linoleic acid and atherosclerosis in rabbits. *Atherosclerosis* **1994**, *108*, 19−25.

(3) Decker, E. A. The role of phenolics, conjugated linoleic acid, carnosine, and pyrroloquinoline quinone as nonessential dietary antioxidants. *Nutr. Rev.* **1995**, *53*, 49−58.

(4) Cook, M. E.; DeVoney, D.; Drake, B.; Pariza, M. W.; Whigham, L.; Yang, M. Dietary control of immune-induced cachexia: Conjugated linoleic acid and immunity. *Adv. Conjugated Linoleic Acid Res.* **1999**, *1*, 226−237.

(5) Turek, J. J.; Li, Y.; Schoenlein, I. A.; Allen, K. G. D.; Watkins, B. A. Modulation of macrophage cytokine production by conjugated linoleic acids is influenced by the dietary n-6:n-3 fatty acid ratio. *J. Nutr. Biochem.* **1998**, *9*, 258−266.

(6) Park, Y.; Albright, K. J.; Storkson, J. M.; Liu, W.; Cook, M. E.; Pariza, M. W. Changes in body composition in mice during feeding and withdrawal of conjugated linoleic acid. *Lipids* **1999**, *34*, 243−248.

(7) Li, Y.; Seifert, M. F.; Ney, D. M.; Grahn, M.; Grant, A. L.; Allen, K. G.; Watkins, B. A. Dietary conjugated linoleic acids alter serum IGF-I and IGF binding protein concentrations and reduce bone formation in rats fed (n-6) or (n-3) fatty acids. *J. Bone Miner. Res.* **1999**, *14*, 1153−1162.

(8) Brodie, A. E.; Manning, V. A.; Ferguson, K. R.; Jewell, D. E.; Hu, C. Y. Conjugated linoleic acid inhibits differentiation of pre- and postconfluent 3T3-L1 preadipocytes but inhibits cell proliferation only in preconfluent cells. *J. Nutr.* **1999**, *129*, 602−606.

(9) Park, Y.; Storkson, J. M.; Albright, K. J.; Liu, W.; Pariza, M. W. Evidence that the *trans*-10,*cis*-12 isomer of conjugated linoleic acid induces body composition changes in mice. *Lipids* **1999**, *34*, 235−241.

(10) DeLany, J. P.; West, D. B. Changes in body composition with conjugated linoleic acid. *J. Am. Coll. Nutr.* **2000**, *19*, 487S-493S.

(11) Moya-Camarena, S. Y.; Van den Heuvel, J. P.; Belury, M. A. Conjugated linoleic acid activates peroxisome proliferator-activated receptor alpha and beta subtypes but does not induce hepatic peroxisome proliferation in Sprague−Dawley rats. *Biochim. Biophys. Acta* **1999**, *1436*, 331−342.

(12) Li, Y.; Watkins, B. A. Conjugated linoleic acids alter bone fatty acid composition and reduce ex vivo prostaglandin E_2 biosynthesis in rats fed n-6 or n-3 fatty acids. *Lipids* **1998**, *33*, 417−425.

(13) Sugano, M.; Tsujita, A.; Yamasaki, M.; Noguchi, M.; Yamada, K. Conjugated linoleic acid modulates tissue levels of chemical mediators and immunoglobulins in rats. *Lipids* **1998**, *33*, 521−527.

(14) Watkins, B. A.; Devitt, A. A.; Feng, S. Designed eggs containing conjugated linoleic acids and omega-3 polyunsaturated fatty acids. *World Rev. Nutr. Diet.* **2001**, *90*, 162−182.

(15) Latour, M. A.; Devitt, A. A.; Meunier, R. A.; Stewart, J. J.; Watkins, B. A. Effects of conjugated linoleic acid. 1. Fatty acid modification of yolks and neonatal fatty acid metabolism. *Poult. Sci.* **2000**, *79*, 817−821.

(16) Cherian, G.; Holsonbake, T. B.; Goeger, M. P.; Bildfell, R. Dietary CLA alters yolk and tissue FA composition and hepatic histopathology of laying hens. *Lipids* **2002**, *37*, 751−757.

(17) Devitt, A.A. Nutraceutical fatty acid modification of chicken egg yolk. M.S. Thesis, Department of Food Science, Purdue University, West Lafeyette, IN, 1999.

(18) National Research Council. *Nutrient Requirements of Poultry*, 9th ed.; National Academy Press: Washington D. C., 1994.

(19) Aluko, R. E.; Mine, Y. Competitive absorption of hen's egg yolk granule lipoprotein and phosvitin in oil-in-water emulsions. *J. Agric. Food Chem.* **1997**, *45*, 4564−4570.

(20) Folch, J.; Lees, M.; Sloane Stanley, G. H. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **1957**, *226*, 497−509.

(21) Li, Y.; Watkins, B.A. Analysis of fatty acids in food lipids. In *Current Protocols in Food Analytical Chemistry*; John Wiley & Sons: New York, 2001; p D1.2.1−D1.2.15.

(22) Yu, L. Conjugated linoleic acid: techniques for analysis and effects on PUFA formation in rat hepatocytes. Ph.D. Thesis, Department of Food Science, Purdue University, West Lafeyette, IN, 1999.

(23) Yu, L.; Adams, D.; Gabel, M. Conjugated linoleic acid isomers differ in their free radical scavenging properties. *J. Agric. Food Chem.* **2002**, *50*, 4135−4140.
(24) Yu, L.; Adams, D.; Watkins, B. A. Comparison of commercial supplements containing conjugated linoleic acids. *J. Food Comp. Anal.* **2003**, *16*, 419−428.
(25) Shenstone, F.S. The gross composition, chemistry, and physical-chemical basis organization of the yolk and white. In *Egg Quality: A Study of the Hen's Egg*; Carter, T. C., Ed.; Oliver and Boyd: Edinburgh, U.K., 1968.
(26) Kircher, H. W. The addition of mercaptants to methyl sterculate and sterculene; an hypothesis concerning the nature of the biological activity exhibited by cyclopropene derivatives. *J. Am. Oil Chem. Assoc.* **1964**, *41*, 4−8.
(27) Reiser, R.; Raju, P. K. The inhibition of saturated fatty acid dehydrogenation by dietary fat containing sterculic and malvalic acids. *Biochem. Biophys. Res. Commun.* **1964**, *17*, 8−11.
(28) Raju, P. K.; Reiser, R. Inhibition of fatty acyl desaturase by cyclopropene fatty acids. *J. Biol. Chem.* **1967**, *242*, 379−384.
(29) Evans, R. J.; Bandemer, S. L.; Anderson, M.; Davidson, J. A. Fatty acid distribution in tissues from hens fed cottonseed oil or Sterculia foetida seeds. *J. Nutr.* **1962**, *76*, 314−319.
(30) Evans, R. J.; Davidson J. A.; Bandemer S. L. Fatty acid and lipid distribution in tissues from hens fed cottonseed oil or *Sterculia foetida* seeds. *J. Nutr.* **1961**, *73*, 282−290.
(31) Shenstone, F. S.; Vickery, J. R. Substances in plant of the order *Malvales* causing pink whites in stored eggs. *Poult. Sci.* **1959**, *38*, 1055−1070.
(32) Lee, K. N.; Pariza, M. W.; Ntambi, J. M. Conjugated linoleic acid decreases hepatic stearoyl−CoA desaturase mRNA expression. *Biochem. Biophys. Res. Commun.* **1998**, *248*, 817−821.
(33) Doberenze, A. D.; Schneider, D. L.; Kurnick, A. A.; Vavich, M. G.; Kemmerer, A. R. Effect of *Sterculia foetida* oil on uptake of water by avian egg yolk. *Proc. Soc. Exp. Biol. Med.* **1960**, *105*, 359−361.

Received for review November 22, 2002. Revised manuscript received August 20, 2003. Accepted September 2, 2003. Approved as Journal Paper Number 16931 of the Purdue University Agricultural Experimental Station.

JF026156V