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Differential Incorporation of Conjugated Linoleic Acid Isomers into Egg Yolk Lipids

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The incorporation pattern of conjugated linoleic acids (CLA) isomers into the egg yolk of hens in relation to that in the diet was studied. Silver-ion high-performance liquid chromatography (Ag-HPLC) was used to separate individual CLA isomers. It was found that the isomeric distribution pattern in the egg yolk lipids was different from that in the dietary fat. Total *cis*/*trans* isomers accounted for 81.2% of total CLA incorporated into the egg yolk, which was in contrast to the value of 92.0% of total CLA in the diet. Total *cis*/*cis* isomers accounted for 3.8% total CLA in the diet but they were 6.6% of the total CLA in the egg yolk lipids. In contrast, total *trans*/*trans* isomers were 12.2% of the total CLA isomers in the egg yolk lipids, whereas they were only 4.2% of total CLA in the diet. The results showed that total *trans*/*trans*-CLA was preferentially incorporated into the egg yolk, whereas the incorporation of total *cis*/*trans*-CLA isomers was partially discriminated. Within each group, the incorporation of individual isomers into the egg yolk lipids was also selective. *cis*-9,*trans*-11/*trans*-9,*cis*-11 and *cis*-10,*trans*-12/*trans*-10,*cis*-12 were the two major isomers in the diet. Ag-HPLC analysis showed that the former was preferentially transferred into the egg yolk compared with the latter. It was observed that supplementation of CLA in the diet of laying hens decreased the concentration of oleic acid (18:1n-9), arachidonic acid (20:4n-6), and docosahexaenoic acid (22:6n-3) but increased that of linolenic acid (18:3n-3), stearic acid (18:0), and palmitic acid (16:0) in the egg yolk, suggesting that CLA may inhibit △6 and △9 desaturases.

KEYWORDS: Conjugated linoleic acids; egg; hens; phospholipids; triglycerides

INTRODUCTION

Conjugated linoleic acids (CLA) are a group of positional and geometric isomers of octadecadienoic acid. Various animal models have demonstrated that CLA are anticarcinogenic (*1*), hypolipidemic (*2*), and antiatherosclerotic (*3*−*4*). CLA have also been shown to enhance immune functions (*5*−*6*) and reduce fat accumulation while they increase muscle and bone mass (*7*−*8*). CLA could behave like an antioxidant in some systems (*9*−*10*), but be a prooxidant in others (*11*−*12*).

CLA in food supply are quantitatively minor, and hence their consumption in humans is only 0.5−1 g/d/person (*13*). In addition to taking CLA supplements, feeding animals with a synthetic CLA mixture should be an alternative to enrich CLA in foods. Supplementation of CLA in the diet of laying hens has led to incorporation of CLA into eggs and changes in yolk fatty acid composition (*14*−*20*). Although the efforts were given to separate individual CLA isomers of egg yolk lipids in previous studies (*14*−*20*), quantification of each isomer incorporated using gas−liquid chromatography (GLC) was impossible because of poor resolution and overlap of CLA isomers. Silver-ion high-performance liquid chromatography (Ag-HPLC) has been shown to resolve CLA isomers well and demonstrated that CLA mixture contained at least 12 isomers (*21*, *22*). Using the Ag-HPLC technique, the present study was carried out further to monitor the incorporation of each CLA isomer into the egg yolk lipids and to ascertain whether there is a selective transfer of CLA isomers.

MATERIALS AND METHODS

Diets. Two CLA mixtures were obtained as gifts from Natural Lipids Ltd, AS, Norway (mixture A) and Bioriginal Food & Science Corp., Saskatoon, SK, Canada (mixture B). The CLA blend supplemented in the diet was a mixture of A and B in a ratio of 1.7 to 1 (wt/wt). A basal chicken diet named poultry breeder was purchased from Glen Forrest Stockfeeds (Western Australia, Australia). According to the supplier, the diet contained 16.0% protein, 4.2% fat, 5.3% fiber, 3.5% calcium, 0.6% phosphorus, 0.3% sodium chloride, and varying amounts

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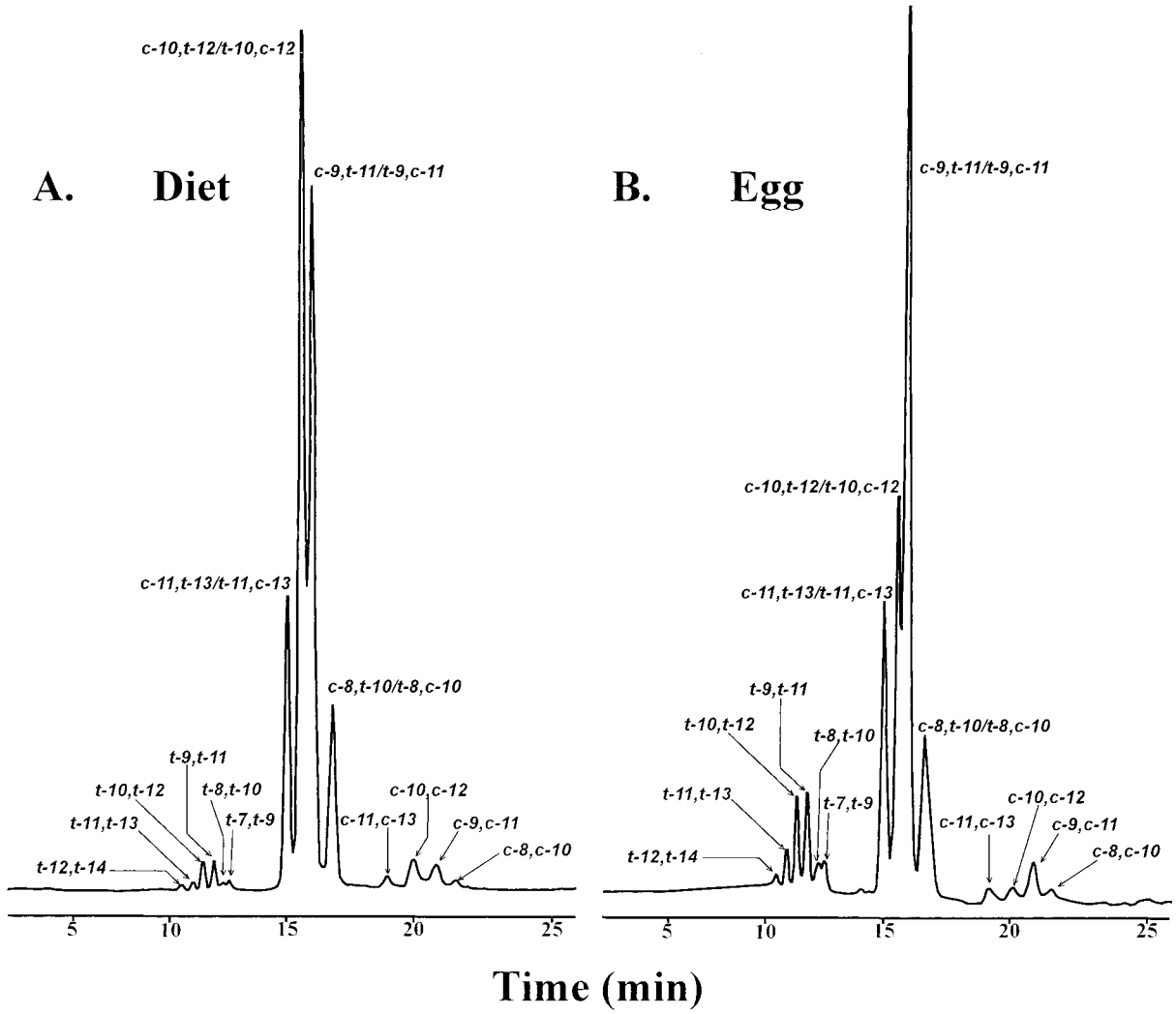

Figure 1. Typical silver-ion high-performance liquid chromatograms of conjugated linoleic acids (CLA), as a form of methyl esters, derived from (A) diet and (B) egg yolk lipids. *c*, *cis*; *t*, *trans*.

of vitamins and other minerals. The control diet was prepared by adding 5% canola oil into the basal diet, whereas the CLA diet was formulated by adding 5% canola oil and 2.2% CLA blend. It was expected that 5% CLA would incorporate into the total egg yolk lipids at 2.2% CLA supplementation in diet according to Du et al. (*20*). The total lipids from both the control and CLA diet were extracted using chloroform and methanol (2:1, vol/vol), and their fatty acid composition was analyzed as described below.

Hens. Fifteen CSIRO Hybrid White Leghorn (*Gallus domesticus*, 20 wks) were divided into two groups and housed (5/cage) in a room at 25 °C with a 12-h light/dark cycle. The first group ($n = 5$) was fed the control diet, whereas the second group ($n = 10$) was fed the CLA diet. The diets were ad libitum given to the hens, and uneaten food was discarded daily. Eggs were collected daily for a period of 4 weeks. The protocol was reviewed and approved by the Committee of Animal Ethics, The Chinese University of Hong Kong.

Fatty Acid Analysis. The diets, 3−5 eggs from the control group, and 7−10 eggs from the CLA group collected on days 0, 6, 11, 16, and 28 were analyzed for their fatty acid composition. Egg yolk was separated from the egg white, and the total lipids derived from the egg yolk were extracted using chloroform/methanol (2:1, vol/vol) containing triheptadecanoic acid as an internal standard. To determine the egg yolk fatty acid composition, acid-catalyzed methylation was used. In brief, the egg yolk lipids (20 mg) were transesterified in 2 mL of 14% BF_3 in methanol under nitrogen at 95 °C for 2 min. Hexane (4 mL) and distilled water (3 mL) were then added and mixed thoroughly. After the mixture was centrifuged, the top hexane layer containing fatty acid methyl esters (FAME) was saved and subjected to gas−liquid chromatographic (GLC) analysis. It was found that the intra-isomerization among the CLA isomers was minimal (<1%) under the present methylation conditions.

To quantify the total egg yolk phospholipids (PL) and triglycerides (TG), L-α-phosphatidylcholine diheptadecanoyl (2 mg) and triheptadecanoate (5 mg) in 1 mLof chloroform as internal standards were added to an aliquot of the egg yolk lipid extract. The mixture was then applied to a 20 × 20 cm thin-layer chomatography (TLC) plate, precoated with 250 μm of silica gel 60 Å (Macherey-Naged, Duren, Germany), to separate different lipid classes. A solvent system of hexane/diethyl ether/acetic acid (80:20:1,v/v/v) was used for development. The bands containing TG and PL were scraped off the plate. The TG and PL were converted to methyl esters and then subjected to GLC analysis.

The FAME mixtures were analyzed on a 100 m × 0.24 mm i.d. SP 2560 fused silica capillary column (Supelco, Inc., Bellefonte, PA) in a HP 5980 Series II gas−liquid chromatograph equipped with a flame-ionization detector and an automated injector (Palo Alto, CA). Column temperature was programmed from 180 to 220 °C at a rate of 1 °C/min and then held for 12 min. Injector and detector temperatures were set at 250 °C and 300 °C, respectively. Hydrogen was used as the carrier gas at a head pressure of 15 psi. The total lipids derived from diets, total egg yolk, and total egg yolk PL and TG were quantified according to the amount of internal standards added into the sample as described previously (*23*).

Ag-HPLC Analysis. The individual CLA methyl esters were separated using an Alltech model 525 HPLC equipped with a ternary pump delivery system as described by Sehat et al. (*21*). In brief, 5 μL of the FAME mixture prepared above (5 μg/mL) in hexane was injected

Table 1. Fatty Acid Composition of Dietary Fat[a]

fatty acids	control	CLA
16:0	5.3 ± 0.1	6.4 ± 0.2
16:1n-7	0.2 ± 0.0	0.2 ± 0.0
18:0	1.6 ± 0.1	2.2 ± 0.3
20:0	0.6 ± 0.1	0.6 ± 0.1
18:1n-9	30.6 ± 0.5	31.2 ± 0.5
18:1n-7	1.7 ± 0.1	1.4 ± 0.1
18:2n-6	18.8 ± 0.4	18.7 ± 0.5
18:3n-3	4.8 ± 0.1	4.6 ± 0.2
CLA	<0.1	16.8 ± 0.5
others	1.1 ± 0.1	1.2 ± 0.1
total	64.7 ± 1.5	83.3 ± 1.1

[a] The values are averages of three determinations, reported in g/kg diet.

onto a 250 × 4.6 mm i.d., 5-μm silver-ion impregnated column (Chrompack, Bridgewater, NJ) via a Rheodyne valve injector. Hexane containing 0.1% acetonitrile was chosen as a mobile phase at a flow rate of 1.0 mL/min. The separated individual CLA methyl esters were monitored at 233 nm using an UVIS-205 spectrophotometer (Alltech, Deerfield, IL). Only the CLA methyl esters were detected; the other FAME were not detectable because they have no absorption at 233 nm. As shown in **Figure 1**, individual CLA isomers were identified according to the Ag-HPLC elution pattern described by Sehat et al. (*21*).

Statistics. Data are expressed as mean ± standard deviation. Where applicable, analysis of variance (ANOVA) was used to statistically evaluate significant differences between the control and the CLA-supplemented group using Sigmastat (Jandel Scientific Software, San Rafael, CA). Differences were considered significant when $p < 0.05$.

RESULTS

Fatty Acid Composition of Dietary Fat. The gas chromatographic analysis showed that the CLA blend contained 81% CLA. As shown in **Table 1**, CLA was absent in the control diet, but it accounted for 16.8 g/kg in the CLA-supplemented diet. The other fatty acids in the two diets were similar except for palmitic acid (16:0) and stearic acid (18:0) which were present in greater amounts in the CLA diet than in the control diet.

Diet Intake and Egg Production. No significant difference in the body weight gain between the two groups of laying hens was observed. The control group had the initial body weight of 1.74 kg and the final body weight of 1.81 kg/hen, whereas the CLA hens initially weighed 1.62 kg and reached 1.68 kg/hen. No differences were observed in diet intakes (control 102, CLA 103 g/d/hen) and production of eggs (0.8 egg/hen/day for both groups). An average egg weight of 53.7 g in the control was slightly greater than that of 51.6 g/egg in the CLA group but the difference was not significant.

Fatty Acid Composition of Total Egg Yolk Lipids. CLA supplementation in the diet significantly altered the fatty acid composition of the egg yolk compared with that of the control diet. As shown in **Figure 2**, CLA supplementation (16.8 g/kg diet) led to incorporation of >3.7% CLA in the total egg yolk lipids after day 11 of supplementation. Compared with the control, the CLA group had a greater amount of linolenic acid (18:3n-3) but it had a lower level of docosahexaenoic acid (22:6n-3) in the egg yolk lipids. Similarly, the CLA hens had a greater amount of linoleic acid (18:2n-6); although a lesser amount of arachidonic acid (20:4n-6) was observed in the CLA hens but it was not significantly different from that of the control value. It was noticed that CLA supplementation led to elevations of stearic acid (18:0) and palmitic acid (16:0) but a reduction in oleic acid (18:1n-9) in the egg yolk lipids.

Table 2. Effects of CLA Supplementation for 28 Days on the Fatty Acid Composition of Egg Yolk Phospholipids and Triglycerides[a]

	phospholipids		triglycerides	
	control	CLA	control	CLA
CLA	0	1.5 ± 0.4	0	2.1 ± 0.6
16:0	27.4 ± 1. 8	27.3 ± 2.6	23.5 ± 1.8	30.8 ± 1.5*
16:1n-9	0.3 ± 0.1	0.2 ± 0.1	1.8 ± 0.4	1.0 ± 0.2*
16:1n-7	0.5 ± 0.1	0.2 ± 0.1*	2.3 ± 0.3	0.8 ± 0.1*
18:0	21.6 ± 0.8	22.8 ± 1.4	4.9 ± 0.6	12.3 ± 1.7*
18:1n-9	23.6 ± 1.0	25.2 ± 1.4	53.6 ± 1.4	37.2 ± 2.3*
18:1n-7	1.1 ± 0.1	1.1 ± 0.3	1.7 ± 0.2	1.7 ± 0.7
18:2n-6	12.4 ± 0.6	13.8 ± 0.8	10.3 ± 1.7	12.4 ± 0.6
20:2n-6	0.1 ± 0.1	0.1 ± 0.1	<0.1	0.1 ± 0.1
20:3n-6	0.2 ± 0.1	0.3 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
20:4n-6	5.9 ± 0.7	4.3 ± 0.6*	0.3 ± 0.1	0.1 ± 0.1*
22:4n-6	0.3 ± 0.1	0.2 ± 0.1	<0.1	<0.1
22:5n-6	0.5 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.1 ± 0.1
18:3n-3	0.4 ± 0.1	0.4 ± 0.1	1.2 ± 0.3	1.7 ± 0.2
22:5n-3	0.3 ± 0.1	0.6 ± 0.2	0.1 ± 0.1	0.1 ± 0.1
22:6n-3	5.4 ± 0.5	2.5 ± 0.4*	0.2 ± 0.1	0.1 ± 0.1*
total (% yolk)	8.0 ± 0.2	7.6 ± 0.3	20.1 ± 2.4	21.6 ± 2.2

[a] Values are means ± SD. * Indicates means at a row for a given lipid class differ significantly compared with the control values, $P < 0.05$.

Fatty Acid Composition of Egg Yolk PL and TG. The fatty acid compositions of egg yolk PL and TG in the CLA group after day 11 were very similar. To simplify the presentation, only data on day 28 are described hereafter. Supplementation of CLA (16.8 g/kg diet) had 1.5% CLA incorporated in egg yolk PL and 2.1% CLA in egg yolk TG (**Table 2**). In the egg yolk PL, the CLA group had lesser amounts of 20:4n-6 and 22:6n-3 than the control, although levels of 18:2n-6 and 18:3n-3 were similar in the two groups. In the egg yolk TG, supplementation of CLA caused elevations of 16:0 and 18:0, but decreased the levels of 16:1n-7, 16:1n-9, 18:1n-9, 20:4n-6, and 22:6n-3.

CLA Isomeric Distribution in the Diet and Egg Yolk Lipids. Ag-HPLC analysis showed that the CLA in the diet contained 92.0% *cis*/*trans* isomers, 4.2% *trans*/*trans* isomers, and 3.8% *cis*/*cis* isomers expressed as percentage of total CLA content (**Table 3**, **Figure 1**). Among four *cis*/*trans* isomers, the cis-*10*,*trans*-12/*trans*-10,*cis*-12 isomers were most abundant (37.9%), followed by *cis*-9,*trans*-11/*trans*-9,*cis*-*11* (31.8%), *cis*-11,*trans*-13/*trans*-11,*cis*-13 (12.8%), and *cis*-8,*trans*-10/*trans*-8,*cis*-*10* (9.6%) in a decreasing order. Within the five *trans*/*trans* isomers, *trans*-9,*trans*-11 and *trans*-10,*trans*-12 were the major isomers, accounting for 1.4% and 1.3%, respectively. *Cis*-*10*,*cis*-12 and *cis*-9,*cis*-11 were the major *cis*/*cis* isomers.

The isomeric distribution pattern in the total egg yolk lipids was different from that in the diet (**Table 3**; **Figure 1**). In the total egg yolk lipids, total *cis*/*trans* isomers accounted for 81.2%, which was in contrast to the value of 92.0% in the diet. In contrast, total *trans*/*trans* isomers in the total egg yolk lipids were 12.3% of the total CLA isomers, whereas they were only 4.2% in the diet (**Table 3**). Total *cis*/*cis* isomers were 6.6% of the total CLA isomers in the egg yolk lipids, whereas they accounted for 3.8% of the total CLA present in the diet.

Within the *cis*,*trans*/*trans*,*cis*-isomers, the total yolk lipids accumulated 41.2% *cis*-9,*trans*-11/*trans*-9,*cis*-11 isomers, which only accounted for 31.8% of total CLA in the diet. In contrast, the total yolk lipids deposited only 16.0% as *cis*-10,*trans*-12/*trans*-10,*cis*-12 isomers, but this isomer accounted for 37.9% of the total CLA isomers in the diet (**Table 3**). The total egg yolk had accumulated greater amounts of individual *trans*,*trans*-isomers including *trans*-9,*trans*-11, *trans*-10,*trans*-12, *trans*-11,*trans*-13, *trans*-8,*trans*-10, *trans*-7,*trans*-9, and *trans*-

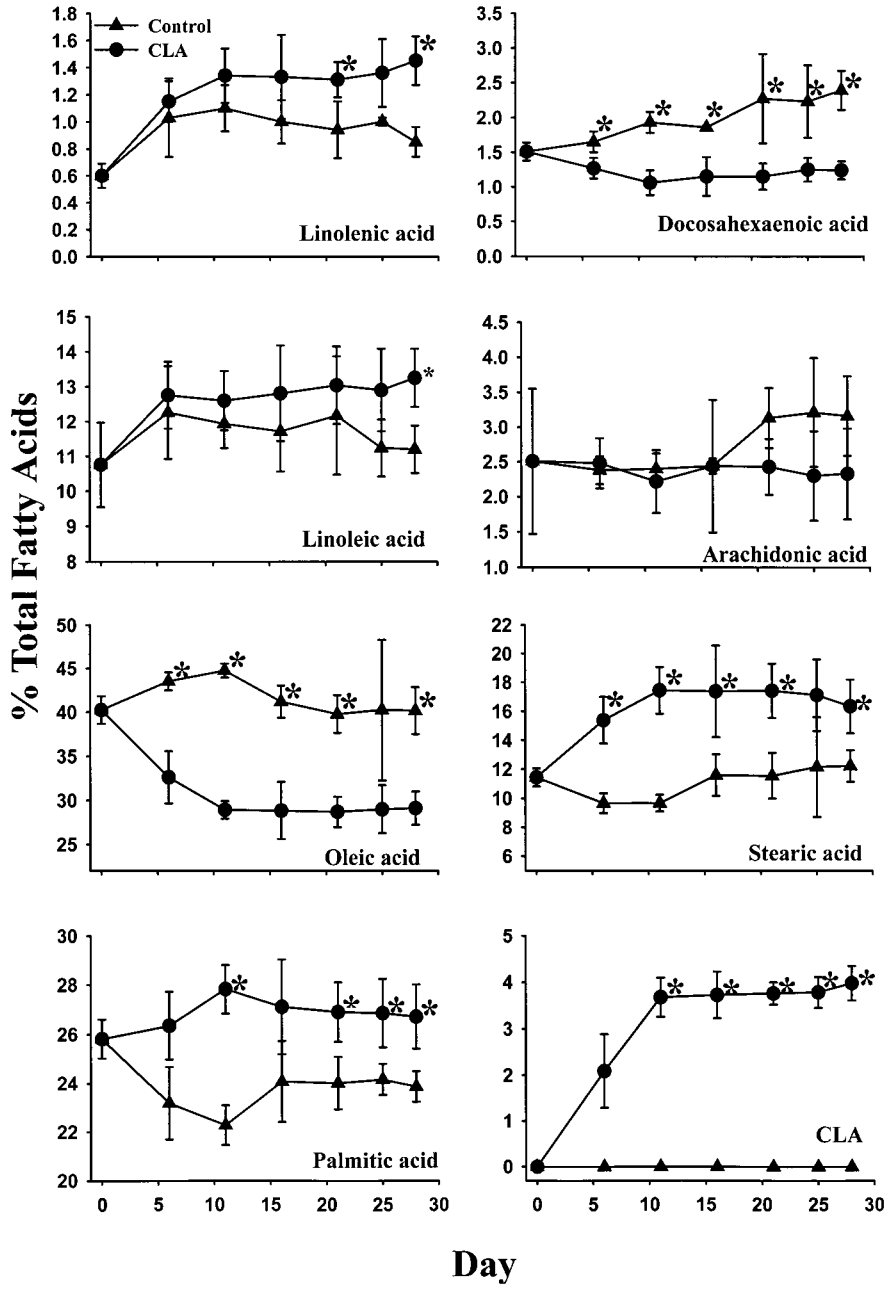

Figure 2. Effect of supplementation of conjugated linoleic acids (CLA) on the fatty acid composition of total egg yolk lipids. The data are expressed as means ± SD, $n = 3–10$ eggs. *Means at the same time point were significantly different between the control hens and CLA groups.

12,*trans*14 compared with the corresponding percentage in the diet. Similarly, the percentages of individual *cis*,*cis*-isomers were greater in the egg yolk lipids than those in the diet except for the *cis*-10,*cis-12* isomer (**Table 3**).

When the isomeric distribution in the egg yolk PL was compared with that in the egg yolk TG, the former had greater proportions of *trans*-10,*trans*-12, *trans*-9,*trans*-11, *cis*-10,*trans*-12/*trans*-10,*cis*-12, and *cis*-9,*trans*-11/*trans*-9,*cis*-11 isomers, but lesser amounts of the isomers *trans*-7,*trans*-9, *cis*-11,*trans*-13/*trans*-11,*cis*-13, and *cis*-8,*trans*-10/*trans*-8,*cis*-10.

DISCUSSION

In agreement with previous reports (*14*−*20*), the results clearly demonstrated that the dietary CLA could be transferred to the egg yolk lipids. Regardless of individual isomers, incorporation of CLA isomers into the egg yolk lipids was significant, reaching a maximum level of 3.7% after day 11 when the diet contained 1.7% CLA (**Figure 2**). This observation is consistent with that reported by Chamruspollert and Sell (*19*), who found that supplementation of CLA at 5.0% CLA led to 11.2% CLA incorporated into the egg yolk lipids. However, the present study clearly showed the transfer of *trans*/*trans*, *cis*/*trans*, and *cis*/*cis* isomers into the egg yolk was a selective process. The *trans*/*trans* CLA isomers appeared to be preferentially incorporated into the egg yolk lipids, whereas the incorporation of *cis*/*trans* CLA isomers was partially discriminated. In the diet, total *trans*/*trans* isomers accounted for 4.2% of total CLA, but in the egg yolk lipids they reached 12.2% of total CLA isomers. In contrast, total *cis*/*trans* isomers were 92.0% in the diet but they were reduced to 81.2% in the egg yolk lipids (**Table 3**).

Incorporation of individual CLA isomers within each group was also selective (**Table 3**). The results demonstrated clearly that two *cis*/*trans* isomers, *cis*-9,*trans*-11/*trans*-9,*cis*-11 and *cis*-

Table 3. Relative Composition (%) of Conjugated Linoleic Acids (CLA) in the Diet, Total Yolk Lipids, Egg Phospholipids, and Egg Triglycerides[a]

CLA isomer	diet	total yolk	phospholipids	triglycerides
trans-12,*trans*-14	0.2 ± 0.1b	0.8 ± 0.5a	0.6 ± 0.3a	0.6 ± 0.3a
trans-11,*trans*-13	0.4 ± 0.2c	1.7 ± 0.5b	3.5 ± 1.3a	2.4 ± 1.0ab
trans-10,*trans*-12	1.3 ± 0.3c	3.1 ± 0.5b	5.3 ± 1.0a	3.6 ± 1.0b
trans-9,*trans*-11	1.4 ± 0.3c	4.2 ± 1.6b	10.2 ± 2.2a	4.6 ± 1.9b
trans-8,*trans*-10	0.5 ± 0.1b	1.3 ± 0.4a	1.1 ± 0.3a	1.8 ± 0.7a
trans-7,*trans*-9	0.6 ± 0.3b	1.2 ± 0.4ab	0.4 ± 0.2b	1.7 ± 0.7a
total *trans*,*trans*-CLA	4.2 ± 0.3c	12.3 ± 3.0b	21.1 ± 2.6a	14.7 ± 1.7b
cis-11,*trans*-13/*trans*-11,*cis*-13	12.8 ± 0.2a	12.9 ± 1.2a	6.2 ± 1.0b	15.1 ± 1.1a
cis-10,*trans*-12/*trans*-10,*cis*-12	37.9 ± 0.7a	16.0 ± 1.6c	19.6 ± 1.0b	12.8 ± 2.0d
cis-9,*trans*-11/*trans*-9,*cis*-11	31.8 ± 0.3c	41.2 ± 2.4ab	43.2 ± 2.1a	37.8 ± 2.4b
cis-8,*trans*-10/*trans*-8,*cis*-10	9.6 ± 0.1c	11.1 ± 0.7b	3.8 ± 0.1d	12.5 ± 1.1a
total *cis*,*trans*-CLA	92.0 ± 0.7a	81.2 ± 3.0b	72.5 ± 3.2c	78.2 ± 2.7bc
cis-11,*cis*-13	0.7 ± 0.1b	1.8 ± 0.6ab	2.2 ± 0.6a	2.5 ± 0.7a
cis-10,*cis*-12	1.5 ± 0.3	1.5 ± 0.4	1.6 ± 0.8	1.3 ± 0.3
cis-9,*cis*-11	1.3 ± 0.2b	2.5 ± 0.3ab	2.8 ± 0.5a	2.5 ± 0.3b
cis-8,*cis*-10	0.4 ± 0.1b	0.9 ± 0.7a	0.3 ± 0.2b	0.6 ± 0.2ab
total *cis*,*cis*-CLA	3.8 ± 0.4b	6.6 ± 0.9a	6.1 ± 0.9a	7.0 ± 1.0a

[a] Values are means ± SD. Letters a,b,c,and d means at a row with different letters differ significantly, $P < 0.05$.

8,*trans*-10/*trans*-8,*cis*-10, were preferentially incorporated into the egg yolk lipids compared with their relative proportions in the diet (**Table 3**, **Figure 1**). All *cis*/*cis* isomers except for *cis*-10,*cis*-12 were accumulated into the egg yolk lipids in proportions more than those in the diet. Similarly, all isomers among the *trans*/*trans* group were selectively incorporated into the egg yolk lipids compared with those in the diet. We are unaware of any studies examining the isomeric distribution in the egg in relation to that in the diet except for that of Chamruspollert and Sell (*14*), who monitored two isomers, namely *cis*-9,*trans*-11/*trans*-9,*cis*-11 and *cis*-10,*trans*-12/*trans*-10,*cis*-12, using the GLC technique and found a preferential incorporation of *cis*-9,*trans*-11/*trans*-9,*cis*-11 over *cis*-10,*trans*-12/*trans*-10,*cis*-12 isomers into the egg yolk. In their study, the analyses of CLA isomers were made on a Supelcowax-10[5] fused silica capillary column, which could not completely resolve the other CLA isomers, and therefore quantification was difficult because some isomers were coeluted. In this regard, identification and quantification of individual CLA isomers became possible when Ag-HPLC was used to separate *trans*/*trans*, *cis*/*trans*, and *cis*/*cis* CLA isomers in the form of methyl esters or free fatty acids (*21*, *22*).

Isomeric distribution into the different lipid classes of egg yolk was also selective (**Table 3**). The egg yolk PL had greater amounts of *cis*-9,*trans*-11/*trans*-9,*cis*-11, *cis*-10,*trans*-12/*trans*-10,*cis*-12, *trans*-9,*trans*-11-9,11 and *trans*-10,*trans*-12 isomers than the egg yolk TG. There are several reports on the distribution of CLA isomers in the animal tissues and in the diet. Kramer et al. (*24*) found there was no difference in distribution of CLA isomers of the commercial CLA fed to the pig and adipose tissue. However, the pig liver PL showed a marked increase of *cis*-9,*trans*-11 CLA compared with that present in the diet. Our previous study found the distribution pattern in the maternal diet was similar to that in the milk (*25*), suggesting that all the CLA isomers in the maternal diet were proportionally absorbed and transferred to mammary glands for milk synthesis. In addition, preferential incorporation of *trans*/*trans* CLA isomers into the liver PL of suckling rats has been reported. The study by Sugano et al. (*26*) measured the lymphatic recovery of CLA isomers and found that *trans*/*trans* isomers were preferentially absorbed, which agrees with the present work. All observations support the view that isomeric distributions of CLA isomers vary not only with the tissues but also with the lipid classes.

No studies to date have addressed the underlying mechanisms for preferential accumulation of some CLA isomers into the egg yolk lipids. Compared with the other groups, the *trans*/*trans* group was selectively incorporated in the egg yolk lipids. It is difficult to use the availability of CLA isomers in the tissue to explain the preferential retention of *trans*/*trans* CLA isomers. Perhaps, the accumulation of *trans*/*trans* CLA isomers was the result of slower metabolism, poor substrates for oxidation, and preferred geometrical insertion in the egg lipids of the laying hens. By the same deduction, the low content of *cis*/*trans* CLA isomers in the egg yolk lipids could be due to rapid metabolism, oxidation, and poor geometrical configuration. Compared with *cis*-10,*trans*-12/*trans*-10,*cis*-12, *cis*-9,*trans*-11/*trans*-9,*cis*-11 showed greater incorporation into the egg yolk lipids. The observation may be explained by the report of Sebedio et al. (*27*) who analyzed the conjugated 20:3 and 20:4 isomers in rats fed a diet containing 180 mg CLA/day and suggested that some *cis*/*trans* CLA isomers were preferentially metabolized to longer chain products via the desaturation and elongation pathway, thus leading tissue to accumulate the isomers that could not be chain-elongated and desaturated.

The impact of CLA supplementation on the other fatty acids of yolk lipids was also noticed with 16:0 and 18:0 being increased but 18:1n-9 being decreased. This is in agreement with the results of Chamruspollert and Sell (*19*), who suggested that CLA inhibited △9 desaturase, which catalyzes the addition of a double bond at the ninth position of 18:0, and hence led to an increase in 18:0 and a decrease in 18:1n-9. The present study also showed that the two precursors of omega-6 and omega-3 families, namely 18:2n-6 and 18:3n-3, were higher in the egg yolk of the CLA group than those in the control, although the control and CLA diets had similar levels of these two fatty acids. In contrast to 18:2n-6 and 18:3n-3, their longer chain metabolites, namely 20:4n-6 and 22:6n-3, were decreased in the egg yolk lipids of the CLA group relative to that of the control hens. The observation was consistent with the results of Chamruspollert and Sell (*19*) and Cook et al. (*28*). By the same reasoning, the CLA probably inhibited △6 desaturase, which is a rate-limiting enzyme catalyzing the reaction of converting 18:2n-6 to 20:4n-6 or 18:3n-3 to 22:6n-3. In fact, some CLA isomers have been shown to compete with 18:3n-3 and 18:2n-6 for △6 desaturase as an inhibitor (*27*, *29*).

It is suggested that *cis*-9,*trans*-11 CLA is the active isomer (*10*, *30*), but several recent reports claim that *trans*-10,*cis*-12

CLA is also biologically potent (*31*, *32*). The present study showed that the transfer of CLA isomers from the diet to the egg yolk was efficient. However, the process was selective; with the *trans*/*trans* isomers being preferentially accumulated, although these isomers were quantitatively minor in both the diet and egg yolk. Regarding *cis*-9,*trans*-11/*trans*-9,*cis*-11 and *cis*-10,*trans*-12/*trans*-10,*cis*-12, the most abundant isomers, the incorporation of the former into the egg yolk was more preferred than the latter.

LITERATURE CITED

(1) Scimeca, J. A. Cancer inhibition in animals. In *Advances in Conjugated Linoleic Acid Research*; Yurawecz, M. P., Mossoba, M. M., Kramer, J. K. G., Pariza, M. W., Nelson, G. J., Eds.; AOCS Press: Champaign, IL, 1999; Volume 1, pp 420−443.

(2) Yeung, C. H. Y.; Yang, L.; Huang, Y.; Wang, J.; Chen, Z. Y. Dietary conjugated linoleic acid mixture affects the activity of intestinal acyl coenzyme A: cholesterol acyltransferase in hamsters. *Br. J. Nutr.* **2000**, *84*, 935−941.

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

(4) Nicolosi, R. J.; Rogers, E. J.; Kritchevsky, D.; Scimeca, J. A.; Huth, P. J. Dietary conjugated linoleic acid reduces plasma lipoproteins and early aortic atherosclerosis in hypercholesterolemic hamsters. *Artery* **1997**, *22*, 266−277.

(5) Cook, M. E.; Miller, C. C.; Park, Y.; Pariza, M. W. Immune modulation by altered nutrient metabolism: Nutritional control of immune-induced growth depression. *Poultry Sci.* **1993**, *72*, 1301−1305.

(6) Miller, C. C.; Park, Y.; Pariza, M. W.; Cook, M. E. Feeding conjugated linoleic acid to animals partially overcomes catabolic responses due to endotoxin injection. *Biochem. Biophys. Res. Commun.* **1994**, *198*, 1107−1112.

(7) Dugan, M. E. R.; Aalhus, J. L.; Schaefer, A.; Kramer, J. K. G. The effects of conjugated linoleic acid on fat to lean repartitioning and feed conversion in pigs. *Can. J. Anim. Sci.* **1997**, *77*, 723−725.

(8) Li, Y.; Watkins, B. A. Conjugated linoleic acids alter bone fatty acid composition and reduce *ex vivo* bone prostaglandin E2 biosynthesis in rats fed n-6 or n-3 fatty acids. *Lipids* **1998**, *33*, 417−425.

(9) Ha, Y. L.; Storkson, J.; Pariza, M. W. Inhibition of benz[*a*]-pyrene-induced mouse forestomach neoplasia by conjugated dienoic derivatives of linoleic acid. *Cancer Res.* **1990**, *50*, 1097−1101.

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

(11) Chen, Z. Y.; Chan, P. T.; Kwan, K. Y.; Zhang, A. Reassessment of the antioxidant activity of conjugated linoleic acids. *J. Am. Oil Chem. Soc.* **1997**, *74*, 749−753.

(12) van den Berg, J. J. M.; Cook, N. E.; Tribble, D. L. Reinvestigation of the antioxidant properties of conjugated linoleic acid. *Lipids* **1995**, *30*, 599−605.

(13) McGuire, W. K.; McGuire, M. A.; Ritzenthaler, K.; Shultz, T. D. Dietary sources and intakes of conjugated linoleic acids. In *Advances in Conjugated Linoleic Acid Research*; Yurawecz, M. P., Mossoba, M. M., Kramer, J. K. G., Pariza, M. W., Nelson, G. J., Eds.; AOCS Press: Champaign, IL, 1999; Volume 1, pp 369−377.

(14) Raes, K.; Huyghebaert, G.; Smet, S. D.; Nollet, L.; Arnouts, S.; Demeyer, D. The deposition of conjugated linoleic acids in eggs of laying hens fed diets varying in fat level and fatty acids. *J. Nutr.* **2002**, *132*, 182−189.

(15) Aydin, R.; Pariza, M. W.; Cook, M. E. Olive oil prevents the adverse effects of dietary conjugated linoleic acid on chick hatchability and egg quality. *J. Nutr.* **2001**, *131*, 800−806.

(16) Schafer, K.; Manner, K.; Sagredos, A.; Eder, K.; Simon, O. Incorporation of dietary linoleic and conjugated linoleic acids and related effects on eggs of laying hens. *Lipids* **2001**, *36*, 1217−1222.

(17) 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. *Poultry Sci.* **2000**, *79*, 817−821.

(18) Jones, S.; Ma, D. W. L.; Robinson, F. E.; Field, C. J.; Clandinin, M. T. Isomers of conjugated linoleic acid (CLA) are incorporated into egg yolk lipids by CLA-fed laying hens. *J. Nutr.* **2000**, *130*, 2002−2005.

(19) Chamruspollert, M.; Sell, J. L. Transfer of dietary conjugated linoleic acid to egg yolks of chickens. *Poultry Sci.* **1999**, *78*, 1138−1150.

(20) Du, M.; Ahn, D. U.; Sell, J. L. Effect of dietary conjugated linoleic acid on the composition of egg yolk lipids. *Poultry Sci.* **1999**, *78*, 1639−1645.

(21) Sehat, N.; Yurawecz, M. P.; Roach, J. A. G.; Mossoba, M. M.; Kramer, J. K. G.; Ku, Y. Silver-ion high-performance liquid chromatographic separation and identification of conjugated linoleic acid isomers. *Lipids* **1998**, *33*, 217−221.

(22) Ostrowska, E.; Dunshea, F. R.; Muralitharan, M.; Cross, R. F. Comparison of silver-ion high-performance liquid chromatographic quantification of free and methylated conjugated linoleic acids. *Lipids* **2000**, *35*, 1147−1153.

(23) Chen, Z. Y.; Menard, R.; Cunnane, S. C. Moderate, selective depletion of linoleate and alpha-linolenate in weight-cycled rats. *Am. J. Physiol.* **1995**, *268*, R498−505.

(24) Kramer, J. K. G.; Sehat, N.; Dugan, M. E. R.; Mossoba, M. M.; Yurawecz, M. P.; Roach, J. A. G.; Eulitz, K.; Aalhus, J. L.; Schaefer, A. L.; Youh, K. U. Distributions of conjugated linoleic acid (CLA) isomers in tissue lipids classes of pigs fed a commercial CLA mixture determined by gas chromatography and silver ion high-performance liquid chromatography. *Lipids* **1998**, *33*, 549−558.

(25) Yang, L.; Yeung, S. K. V.; Huang, Y.; Wang, H. Q.; Chen, Z. Y. Preferential incorporation of trans, trans-conjugated linoleic acid isomers in the liver of suckling rats. *Br. J. Nutr.* **2002**, *87*, 253−260.

(26) Sugano, M.; Tsujita, A.; Yamasaki, A.; Yamada, K.; Ikeda, I.; Kritchevsky, D. Lymphatic recovery, tissue distribution, and metabolic effects of conjugated linoleic acid in rats. *J. Nutr. Biochem.* **1997**, *8*, 38−43.

(27) Sebedio, J. L.; Juaneda, P.; Dobson, G.; Ramilison, I.; Martin, J. C.; Chardigny, J. M.; Christie, W. W. Metabolites of conjugated isomers of linoleic acids (CLA) in the rat. *Biochim. Biophys. Acta* **1997**, *1345*, 5−10.

(28) Cook, M. E.; Pariza, M. W.; Lee, K. N.; Wentworth, B. C. Method for controlling bird populations. U.S. Patent 5,504,114, 1996.

(29) Belury, M. A.; Kempa-Steczko, A. Conjugated linoleic acid modulates hepatic lipid composition in mice. *Lipids* **1997**, *32*, 199−204.

(30) Knekt, P.; Jarvinen, R.; Seppanen, R.; Pukkala, E.; Aromaa, A. Intake of dairy products and the risk of breast cancer. *Brit. J. Cancer* **1996**, *73*, 687−691.

(31) Leung, Y. H.; Liu, R. H. *Trans*-10, *cis*-12-conjugated linoleic acid isomer exhibits stronger oxyradical scavenging capacity than *cis*-9, *trans*-11 conjugated linoleic acid isomer. *J. Agric. Food Chem.* **2000**, *48*, 5469−5475.

(32) Lin, Y.; Kreeft, A.; Schuurbiers, J. A.; Draijer, R. Different effects of conjugated linoleic acid isomers on lipoprotein lipase activity in 3T3 adipocytes. *J. Nutr. Biochem.* **2001**, *12*, 183−189.

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