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Isomeric Distribution of Conjugated Linoleic Acids (CLA) in the Tissues of Layer Hens Fed a CLA Diet

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The isomeric distribution of conjugated linoleic acids (CLA) in the tissue lipids of hens in relation to that in the diet was examined. Silver-ion high-performance liquid chromatography was used to quantify individual CLA isomers in total tissue lipids, phospholipids, and triacylglycerols. It was found that the deposition of CLA isomers in hen tissues was selective. All tissues including serum, liver, heart, kidney, abdominal fat, and leg and breast muscles had lesser amounts of total *cis/trans* isomers ranging from 75.87 to 89.13% of total CLA, which was in contrast to the value of 92% of total CLA in the dietary lipids. Total *trans/trans* isomers in all tissue lipids ranging from 6.11 to 18.02% of total CLA were greater than that in the diet (4.19%). Among the individual *trans/trans* isomers, all tissues except for adipose tissue and brain incorporated greater amounts of *t*-12,*t*-14−18:2, *t*-11,*t*-13−18:2, *t*-10,*t*-12−18:2, *t*-9,*t*-11−18:2, and *t*-18,*t*-10−18:2 compared with the values of the diet. Within the *cis/trans* group, lesser amounts of *c*-10,*t*-12/*t*-10,*c*-12−18:2 were found to incorporate into all tissues compared with the value of the diet. Serum and liver had higher percentages of *c*-9,*t*-11/*t*-9,*c*-11, whereas the other tissues had similar levels of this isomer compared with that of the diet. It was also observed that supplementation of CLA in the diet of layer hens decreased the concentration of docosahexaenoic acid (22:6*n*−3) in all of the tissue lipids. It is concluded that dietary CLA can transfer to the tissue but that incorporation of CLA isomers into the tissue is selective in hens.

KEYWORDS: Conjugated linoleic acids; hens; phospholipids; triglycerides

INTRODUCTION

Conjugated linoleic acids (CLA) have been extensively studied over the past two decades for several possible health benefits in relation to their supplementation in the diet, including being anticarcinogenic (*1*), hypolipidemic (*2*), and antiatherosclerotic (*3*, *4*). CLA have been demonstrated to enhance immune functions (*5*, *6*) and reduce fat accumulation while increasing muscle and bone mass (*7*, *8*). However, the claim that CLA are antioxidants remains inconclusive because they are antioxidative in some systems (*9*, *10*) but prooxidative in others (*11*, *12*). Dietary CLA predominately originate from dairy products via biohydrogenation of α-linolenic acid and isomerization of linoleic acids by rumen microorganisms (*13*−*15*). In addition, a minor amount of CLA is also biosynthesized from desaturation of *trans*-11-octadeceenoic acid catalyzed by Δ-9 desaturase in ruminants, rodents, and humans (*16*−*19*). Low concentrations of CLA also occur in the lipids of human blood, tissue, and breast milk (*20*, *21*). However, the proportion from diets or endogenous biosynthesis remains unknown. Commercially, CLA can be prepared by alkali isomerization of linoleic acid (*22*) and dehydration of ricinoleic acid in castor oil (*23*, *24*).

Silver-ion high-performance liquid chromatographic (Ag-HPLC) analysis has demonstrated that CLA contain at least 12 isomers (*25*, *26*). Information on the biological activities of each CLA isomer is very limited. Some evidence supports *c*-9,*t*-11−18:2 as the active isomer (*10*, *27*), whereas several reports suggest that *t*-10,*c*-12−18:2 is biologically more potent than *c*-9,*t*-11−18:2 (*28*, *29*). CLA in food supply are quantitatively minor, and hence their consumption in humans is only 0.5−1 g/day/person (*13*). In addition to taking CLA supplements, feeding animals a synthetic CLA mixture should be an alternative to enrich CLA in foods. Supplementation of CLA in the feed has led to the incorporation of CLA in eggs of hens and in pigs (*31*−*34*). Although efforts were made to quantify individual CLA isomers of egg yolk and tissue lipids in previous studies (*31*−*33*), separation of these isomers incorporated into the tissue was impossible because of poor resolution and overlap of CLA isomers using gas−liquid chromatography (GLC). By using Ag-HPLC, we were able to demonstrate that isomeric distribution of CLA in rat milk was similar to that in maternal diet (*35*),

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but incorporation of CLA isomers into rat liver and egg yolk lipids was selective, with *trans/trans* isomers being preferentially incorporated (*36*). The present study was carried out to further examine the incorporation pattern of CLA isomers into various tissues of layer hens fed a 2% CLA diet. It was found that incorporation of CLA isomers into tissues was also selective.

MATERIALS AND METHODS

Hens and Diets. 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% salt, and varying amounts of vitamins and other minerals. Two CLA mixtures were obtained from Natural Lipids Ltd., AS, Norway (mixture A) and Bioriginal Food and 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 (w/w). 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.

Fifteen CSIRO Hybrid White Leghorn (*Gallus domesticus*, $n = 15$) were divided into two groups and housed ($n = 5$/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 fiven ad libitum to the hens, and uneaten food was discarded daily. Eggs were collected daily for a period of 4 weeks. On day 28, blood was collected from the vein of a wing into a syringe; after clotting, serum was separated from whole blood. All hens were then anesthetized under carbon dioxide and then killed on day 28. Liver, heart, kidney, brain, abdominal fat, and breast and left leg muscles were removed, washed with 0.9% saline, and frozen at −80 °C. The protocol was reviewed and approved by the Committee of Animal Ethics, The Chinese University of Hong Kong.

Lipid Analysis. Total lipids of tissues (1 g) were extracted using 50 mL of chloroform/methanol (2:1, v/v) containing 0.02% butylated hydroxytoluene as an antioxidant. To quantify the total lipids in tissues, heptadecanoic acid (2 mg) in methanol was added to an aliquot of the tissue lipid extract (20 mg of lipids). The total tissue lipids were transesterified to fatty acid methyl esters (FAME) in 2 mL of 14% BF_3 in methanol under nitrogen gas for 2 min at 95 °C. To quantify the total triacylglycerols (TG) and phopholipids (PL), triheptadecanoin (2 mg) and l-phosphatidylcholine diheptadecanoyl (2 mg) in 1 mL of chloroform (Sigma Chemical) were added as internal standards to an aliquot of the total lipids extract (30 mg). Lipid classes were separated by thin-layer chromatography (20 × 20 cm plates precoated with 0.25 mm silica gel 60A, Macherey-Nagel, Duren, Germany) using a developing solvent system of hexane/diethyl ether/acetic acid (80:20:1, v/v/v). TG and PL bands were recovered from the TLC plate and converted to the corresponding methyl esters using 2 mL of 14% BF_3 in methanol under nitrogen at 95 °C for 2 min. Four milliliters of hexane and 3 mL of distilled water were then added and mixed thoroughly. After centrifugation, the top hexane layer containing FAME was saved and subjected to gas−liquid chromatographic (GLC) analysis. It was found that the intraisomerization of CLA species was minimal (<1%) under the present methylation conditions.

The FAME mixtures were analyzed on a flexible silica capillary column (SP 2560, 100 m × 0.25 mm, i.d.; Supelco, Inc., Bellefonte, PA) in an 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 and 300 °C, respectively. Hydrogen was used as the carrier gas at a head pressure of 100 kPa. The total lipids, PL, and TG were quantified according to the amount of internal standards added during the extraction (*37*).

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. (*17*). In brief, 5 μL of FAME mixture prepared above (5 μg/mL) in hexane was injected onto a silver-ion impregnated column (4.6 mm i.d. × 250 mm stainless, 5 μm, 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 ester isomers were monitored at 233 nm (UVIS-205, Alltech, Deerfield, IL). Only CLA methyl ester isomers have absorption, whereas other fatty acids containing no conjugated double bonds have no absorption. Individual CLA isomers were identified according to the Ag-HPLC eluting pattern described by Sehat et al. (*25*). **Figure 1** illustrates typical chromatograms of CLA isomeric distributions in the diet and liver lipid extract.

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

Table 1. Fatty Acid Composition of Dietary Fat

	control		CLA supplementation	
fatty acid	g/kg of diet	% of total fatty acids	g/kg of diet	% of total fatty acids
16:0	5.3	8.2	6.4	7.6
18:0	0.2	0.3	0.2	0.2
20:0	1.7	2.5	2.2	2.6
16:1*n*–7	0.6	0.9	0.6	0.7
18:1*n*–9	30.6	47.3	31.2	37.6
18:1*n*–7	1.7	2.6	1.4	1.7
18:2*n*–6	18.8	29.1	18.7	22.5
18:3*n*–3	4.8	7.4	4.6	5.5
CLA	<0.1	<0.1	16.8	20.2
others	1.1	1.7	1.2	1.4
total	64.7	100	83.3	100

Table 2. Body and Tissue Weights of Hens Fed the Control and CLA-Supplemented Diet[a]

	control		CLA-supplemented	
	initial	final	initial	final
body wt (kg)	1.7 ± 0.2	1.8 ± 0.2	1.6 ± 0.1	1.7 ± 0.1
liver (g)	ND[b]	52.8 ± 9.0	ND	49.1 ± 6.4
kidney (g)	ND	3.1 ± 0.7	ND	3.0 ± 0.5
brain (g)	ND	2.8 ± 0.3	ND	2.7 ± 0.2
heart (g)	ND	7.4 ± 1.0	ND	7.2 ± 0.6

[a] Values are means ± SD, $n = 5$–10. [b] ND, not determined.

RESULTS

Fatty Acid Composition of Dietary Fat. Total CLA in the diet were quantified using GLC. The result showed that the CLA blend contained 81% CLA. The control diet has no CLA, whereas the CLA diet had 16.8 g/kg of diet (**Table 1**). When the fatty acid composition was expressed as grams per kilogram of diet, the other fatty acids in the two diets were similar except for palmitic acid (16:0) and stearic acid (18:0), being greater in the CLA diet than in the control diet.

Diet Intake, Tissue Weights, and Egg Production. The body weight gain between the two groups of hens was not significant (**Table 2**). No significant differences in weights of the liver, kidney, brain, and heart were observed. Neither was the diet intake (control, 102 g/day/hen; CLA, 103 g/day/hen). Part of the study on egg production and fatty acid composition of yolk lipids has been reported elsewhere (*36*). In brief, production of eggs was 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 Tissue Lipids. Dietary CLA deposited in all tissues except for brain. CLA supplementation

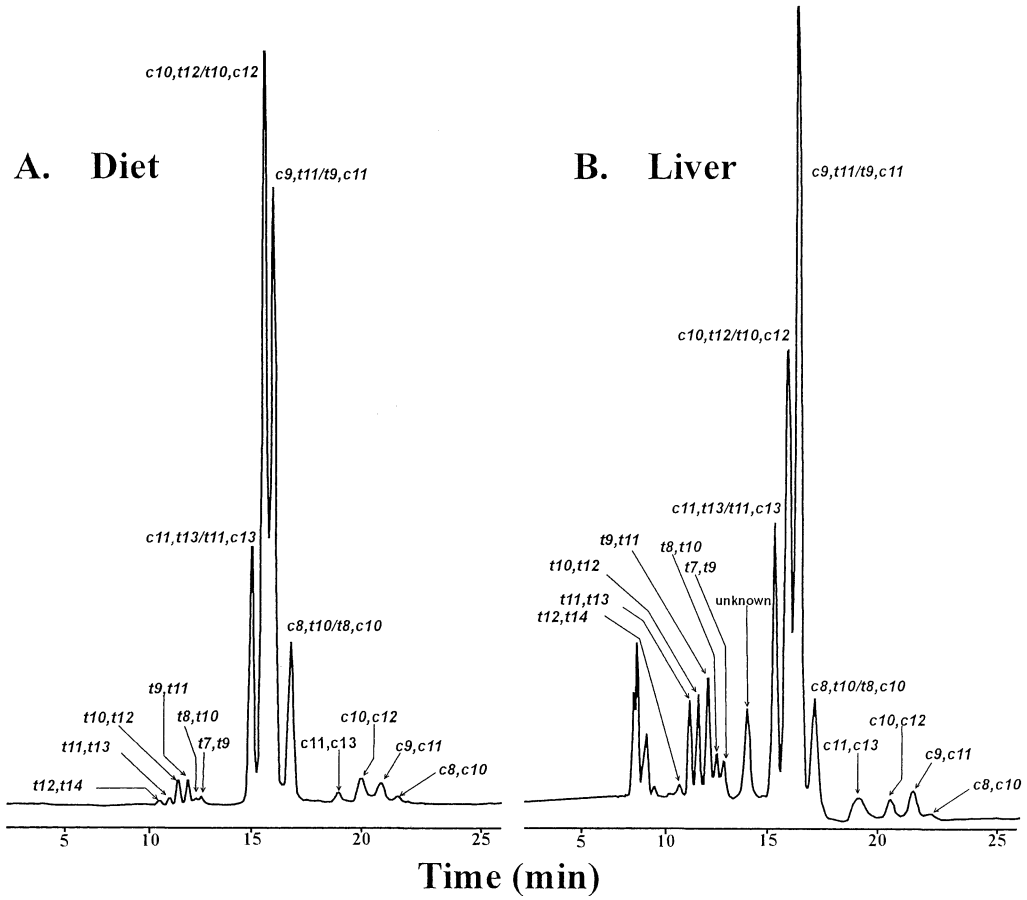

Figure 1. Typical silver-ion high-performance liquid chromatograms of CLA as a form of methyl esters derived from lipids of the diet and liver of hens fed the CLA-supplemented diet. Separation was performed in a silver-ion impregnated Chrompack analytical column (4.6 mm i.d. × 250 mm), and hexane containing 0.1% acetonitrile was used as a mobile phase at a flow rate of 1 mL/min. *c*, cis; *t*, trans.

in the diet significantly altered the fatty acid composition of the tissue lipids compared with the control diet. CLA supplementation led to incorporation of >2.79% CLA in the total lipids of the liver. Compared with the control, the CLA group had a greater amount of stearic acid (18:0) but lower levels of docosahexaenoic acid (22:6*n*−3), oleic acid (18:1*n*−9), and palmitoleic acid (16:1*n*−7) in the liver lipids. Although a lesser effect of CLA supplementation on other fatty acids in the other tissues was observed, the CLA group was consistently lower in 22:6*n*−3 compared with the control group (**Tables 3** and **4**).

CLA Isomeric Distribution in the Diet and Tissue Lipids. Ag-HPLC analysis showed that the CLA blend added into the diet contained at least 14 isomers (**Figure 1**). Total *cis/trans* isomers accounted for 92% followed by total *trans/trans* isomers (4.19%) and total *cis/cis* isomers (3.81%) as expressed as percentages of total CLA content (**Table 5**). The isomeric distribution pattern in the total lipids of all tissue analyzed was different from that in the diet (**Table 5**; **Figure 1**). All tissues had lesser amounts of total *cis/trans* isomers (75.87−89.13%), which was in contrast to the value of 92.00% in the diet ($p < 0.05$). In contrast, total *trans/trans* isomers in all tissue lipids were greater than that in the diet (**Table 5**). Among the individual *trans/trans* isomers, all total tissues except for adipose tissue incorporated greater amounts of *t*-12,*t*-14−18:2, *t*-11,*t*-13−18:2, *t*-10,*t*-12−18:2, *t*-9,*t*-11−18:2, and *t*-18,*t*-10−18:2 compared with the value of the diet (**Table 5**). Within the *cis/trans* group, all tissues except serum and liver accumulated a greater percentile of *c*-11,*t*-13/*t*-11,*c*-13−18:2 compared with the diet. In contrast, a lesser amount of *c*-10,*t*-12/*t*-10,*c*-12−18:2 was incorporated into all tissues analyzed compared with the value of the diet (**Table 5**). Serum and liver had higher percentages of *c*-9,*t*-11/*t*-9,*c*-11−18:2, whereas the other tissues had similar levels of incorporation of this isomer compared with that of the diet. The percentage of *c*-8,*t*-10/*t*-8,*c*-10−18:2 in all tissue lipids was similar to that of the diet except for the adipose tissue that had a greater percentage of *c*-8,*t*-10/*t*-8,*c*-10−18:2 than the diet. Within *cis/cis* isomers, all tissues except for breast muscle had a greater percentage of *c*-11,*c*-13−18:2 than the diet. Other *cis/cis* isomers did not have a distinct pattern in the tissue compared with those in the diet (**Table 5**).

All tissue PL incorporated a greater amount of total *trans/trans* isomers (10.59−20.04%) compared with 4.19% in the diet (**Table 6**). In contrast, lesser amounts of total *cis/trans* isomers (75.71−87.03%) incorporated into all tissue PL compared with that in the diet (92%). All tissue PL except for the liver and leg muscle accumulated a higher percentage of total *cis/cis* isomers compared with that in the diet. Within individual *trans/trans* isomers, higher percentages of *t*-11,*t*-13−18:2 and *t*-9,*t*-11−18:2 were observed to incorporate into all tissue lipids compared with the value for the diet lipids (**Table 6**). A similar trend was observed for *t*-12,*t*-14−18:2 in all tissue PL except for breast muscle. The tissue PL levels of *t*-8,*t*-10−18:2 and *t*-7,*t*-9−18:2 were not significantly different from that of the diet except for kidney and adipose tissue. Among the individual *cis/trans* isomers, *c*-11,*t*-13/*t*-11,*c*-13−18:2 incorporated preferentially into heart, kidney, adipose tissue, and leg and breast muscles but its incorporation into the liver and serum PL was partially discriminated compared with the value of the diet (**Table 6**). All tissues consistently had lesser *c*-10,*t*-12/*t*-10,*c*-12−18:2 incorporated into PL compared with the value of the diet. Three

Table 3. Fatty Acid Composition (Percent of Total Fatty Acids) of Liver, Heart, Brain, and Kidney of Hens Fed the Control and CLA-Supplemented Diets[a]

	liver		heart		kidney		brain	
fatty acid	control	CLA	control	CLA	control	CLA	control	CLA
14:0	0.31 ± 0.05	0.45 ± 0.17	0.90 ± 0.11	0.75 ± 0.26	0.43 ± 0.08	0.48 ± 0.06	0.53 ± 0.13	0.46 ± 0.11
16:0	22.11 ± 1.62	24.87 ± 1.69	20.45 ± 1.74	18.59 ± 1.74	19.44 ± 0.38	18.58 ± 0.93	24.77 ± 1.40	24.20 ± 2.03
18:0	12.06 ± 2.39	20.24 ± 1.99*	13.16 ± 2.03	10.76 ± 1.58	18.05 ± 0.82	18.84 ± 2.35	20.25 ± 0.74	19.94 ± 1.58
16:1*n*–7	1.92 ± 0.79	0.63 ± 0.10*	1.91 ± 0.38	1.57 ± 0.23	0.95 ± 0.20	0.46 ± 0.08*	1.07 ± 0.17	0.75 ± 0.42
16:1*n*–9	0.95 ± 0.06	0.63 ± 0.12	0.67 ± 0.12	0.57 ± 0.14	0.53 ± 0.01	0.29 ± 0.10	0.69 ± 0.06	0.52 ± 0.22
18:1*n*–9	42.33 ± 3.98	28.50 ± 2.69*	33.36 ± 3.71	34.48 ± 1.79	24.40 ± 1.56	22.75 ± 3.22	21.30 ± 1.31	21.09 ± 3.48
18:2*n*–6	12.19 ± 1.64	13.51 ± 0.74	17.93 ± 1.43	18.14 ± 0.48	17.09 ± 1.64	18.83 ± 1.33	0.75 ± 0.18	0.96 ± 0.21
20:2*n*–6	0.19 ± 0.15	0.09 ± 0.07	0.10 ± 0.02	0.10 ± 0.03	0.66 ± 0.09	0.44 ± 0.25	0.73 ± 0.11	0.30 ± 0.12*
20:3*n*–6	0.42 ± 0.27	0.50 ± 0.14	0.64 ± 0.20	0.25 ± 0.13	2.76 ± 0.46	2.31 ± 0.57	0.41 ± 0.15	0.89 ± 0.41*
20:4*n*–6	3.33 ± 1.19	4.63 ± 1.33	6.02 ± 1.55	4.06 ± 1.81	10.14 ± 0.46	9.11 ± 1.27	11.17 ± 0.40	11.27 ± 1.25
22:4*n*–6	0.70 ± 0.53	0.18 ± 0.05	0.32 ± 0.12	0.15 ± 0.05	0.27 ± 0.09	0.45 ± 0.11	3.37 ± 0.85	3.39 ± 0.49
22:5*n*–6	0.26 ± 0.12	0.20 ± 0.05	0.42 ± 0.15	0.32 ± 0.17	0.44 ± 0.10	0.54 ± 0.17	1.26 ± 0.46	1.33 ± 0.59
18:3*n*–3	0.96 ± 0.25	0.88 ± 0.22	1.38 ± 0.32	1.88 ± 0.36	0.69 ± 0.13	0.99 ± 0.23	0.07 ± 0.02	0.09 ± 0.05
20:5*n*–3	0.17 ± 0.02	0.39 ± 0.14	0.48 ± 0.20	0.65 ± 0.42	1.10 ± 0.11	1.16 ± 0.33	0.57 ± 0.23	1.41 ± 0.20
22:5*n*–3	0.11 ± 0.06	0.33 ± 0.06	0.29 ± 0.07	0.23 ± 0.14	0.37 ± 0.05	0.52 ± 0.11	0.32 ± 0.13	0.35 ± 0.13
22:6*n*–3	1.98 ± 0.76	1.17 ± 0.21*	1.43 ± 0.31	0.77 ± 0.34*	2.70 ± 0.39	1.93 ± 0.40*	12.61 ± 0.87	13.08 ± 1.92
CLA	ND	2.79 ± 0.32	ND	2.62 ± 0.28	ND	2.32 ± 0.42	ND	ND
total lipids (g/100 g of tissue)	3.42 ± 0.38	3.23 ± 0.36	2.48 ± 0.29	2.62 ± 0.51	1.91 ± 0.28	2.06 ± 0.44	2.72 ± 0.59	2.45 ± 0.23

[a] Values are means ± SD, $n = 5–10$. ND = not detectable. * = differs significantly from the value of the control, $p < 0.05$.

Table 4. Fatty Acid Composition (Percent of Total Fatty Acids) of Abdominal Fat and Leg and Breast Meats of Hens Fed the Control and CLA-Supplemented Diets[a]

	fat		leg		breast	
fatty acid	control	CLA	control	CLA	control	CLA
14:0	0.70 ± 0.06	0.70 ± 0.04	0.84 ± 0.19	1.34 ± 0.20	0.27 ± 0.06	0.84 ± 0.19
16:0	19.68 ± 1.53	19.56 ± 1.20	17.76 ± 0.78	16.51 ± 0.46	21.26 ± 1.02	21.11 ± 1.62
18:0	5.25 ± 0.89	6.31 ± 0.95	14.71 ± 0.65	15.51 ± 0.67	13.89 ± 0.30	11.94 ± 1.12
16:1*n*–7	4.58 ± 1.60	3.07 ± 0.82	1.62 ± 0.37	1.34 ± 0.76	1.52 ± 0.19	1.18 ± 0.43
16:1*n*–9	0.42 ± 0.22	0.51 ± 0.04	0.51 ± 0.06	0.37 ± 0.04	0.35 ± 0.08	0.38 ± 0.09
18:1*n*–9	46.55 ± 0.53	42.67 ± 2.58	26.32 ± 1.33	25.88 ± 2.42	32.00 ± 1.44	32.50 ± 1.87
18:2*n*–6	18.45 ± 1.64	18.06 ± 0.84	20.33 ± 1.28	20.01 ± 1.25	12.29 ± 1.26	15.77 ± 1.70*
20:2*n*–6	0.10 ± 0.02	0.15 ± 0.06	0.18 ± 0.05	0.13 ± 0.05	ND	ND
20:3*n*–6	0.20 ± 0.09	0.39 ± 0.17	0.33 ± 0.04	0.32 ± 0.12	2.23 ± 0.68	0.42 ± 0.16
20:4*n*–6	0.32 ± 0.08	0.24 ± 0.06	14.70 ± 2.66	13.45 ± 2.11	9.14 ± 0.56	8.52 ± 1.62
22:4*n*–6	0.10 ± 0.04	0.10 ± 0.03	0.39 ± 0.08	0.34 ± 0.10	ND	ND
22:5*n*–6	0.20 ± 0.09	0.28 ± 0.10	0.27 ± 0.02	0.80 ± 0.02	1.26 ± 0.36	0.69 ± 0.16
18:3*n*–3	2.86 ± 0.30	3.09 ± 0.39	0.87 ± 0.18	1.18 ± 0.36	0.84 ± 0.15	1.42 ± 0.37
20:5*n*–3	0.27 ± 0.08	0.33 ± 0.08	0.13 ± 0.06	0.18 ± 0.01	1.21 ± 0.46	0.70 ± 0.41
22:5*n*–3	0.12 ± 0.04	0.24 ± 0.08	0.27 ± 0.02	0.80 ± 0.02	0.70 ± 0.18	0.53 ± 0.11
22:6*n*–3	0.19 ± 0.04	0.10 ± 0.04*	0.51 ± 0.10	0.29 ± 0.11*	2.45 ± 0.22	1.98 ± 0.18*
CLA	ND	4.10 ± 0.86	ND	2.03 ± 0.17	ND	2.28 ± 0.48
total lipids (g/100 g of tissue)	65.61 ± 3.71	66.62 ± 4.81	2.43 ± 0.27	2.77 ± 10.13	1.69 ± 0.21	1.66 ± 0.19

[a] Values are means ± SD, $n = 5–10$. ND = not detectable. * = differs significantly from the value of the control, $p < 0.05$.

tissues including serum, liver, and breast muscle PL had higher percentages of *c*-9,*t*-11/*t*-9,*c*-11−18:2 than in the diet, whereas other tissue PL had incorporation of this isomer in a proportion not different from that in the diet. Except for the adipose tissue, all tissue PL had lower a proportion of *c*-8,*t*-10/*t*-8,*c*-10−18:2 than the diet. No characteristic pattern of incorporation into the tissue PL could be observed for individual *cis/cis* isomers. Incorporation of CLA isomers into the tissue TG was similar to that in the tissue PL with total *trans*/*trans* being preferentially incorporated and *cis*/*trans* CLA isomers being partially discriminated (**Table 7**). To simplify the presentation, only data for major *cis*/*trans* isomers are described. *c*-11,*t*-13/*t*-11,*c*-13−18:2 incorporated selectively into the tissue TG. The reverse was seen for *c*-10,*t*-12/*t*-10,*c*-12−18:2. Incorporation of *c*-9,*t*-11/*t*-9,*c*-11−18:2 into the tissue TG was preferential in serum and liver, but in other tissues TG was in proportion similar to that in the diet.

DISCUSSION

The results clearly demonstrated that the dietary CLA could transfer to all tissues except for the brain, where CLA appears to be incapable of passing the blood−brain barrier. Incorporation of CLA varied with the tissues, ranging from 2.03% (leg muscle) to 4.10% (adipose tissue) of total lipids when the diet contained 16.8 g of CLA/kg of diet (**Tables 3** and **4**). At a similar level of CLA supplementation, incorporation of CLA in the egg yolk lipid reached 3.7% (*36*). Supplementation of CLA at 50 g/kg of diet led to 11.2% CLA incorporated into the egg yolk lipids (*31*). Our previous study demonstrated that supplementation of 14.6 g of CLA/kg of maternal diet could lead to 8.6% CLA

Table 5. Relative Composition of Conjugated Linoleic Acids in Total Lipids of Diet and Tissues of Hens Fed the CLA-Supplemented Diets[a]

CLA isomer	diet	serum	liver	heart	kidney	adipose	leg	breast
*t*12,*t*14	0.17 ± 0.07c	0.41 ± 0.18b	0.53 ± 0.28ab	0.41 ± 0.15b	0.86 ± 0.22a	0.20 ± 0.05c	0.60 ± 0.27ab	0.72 ± 0.24ab
*t*11,*t*13	0.38 ± 0.19c	2.66 ± 1.06ab	1.59 ± 1.09b	2.85 ± 0.78ab	3.77 ± 1.93ab	0.71 ± 0.24c	3.29 ± 1.63ab	4.16 ± 1.16a
*t*10,*t*12	1.27 ± 0.30c	4.24 ± 1.33a	2.28 ± 0.91b	2.81 ± 0.21b	3.45 ± 1.04a	1.98 ± 0.29c	3.60 ± 1.06ab	3.97 ± 1.07ab
*t*9,*t*11	1.35 ± 0.28c	6.94 ± 2.11a	2.68 ± 1.08bc	3.49 ± 0.08b	5.79 ± 1.30a	1.82 ± 0.33c	5.27 ± 1.60a	7.28 ± 1.72a
*t*8,*t*10	0.45 ± 0.26c	2.02 ± 0.37a	1.58 ± 1.12ab	0.78 ± 0.17b	1.40 ± 0.66ab	0.67 ± 0.13bc	1.09 ± 0.45b	1.29 ± 0.34b
*t*7,*t*9	0.58 ± 0.34b	1.74 ± 0.33a	1.32 ± 0.52ab	0.90 ± 0.24b	1.53 ± 0.62a	0.73 ± 0.13b	0.64 ± 0.49b	0.59 ± 0.19b
total *t*,*t*-CLA	4.19 ± 0.34d	18.01 ± 5.0a	9.98 ± 3.15b	11.25 ± 1.12b	16.81 ± 3.58ab	6.11 ± 0.66c	14.49 ± 4.65ab	18.02 ± 3.01a
*c*11,*t*13/*t*11,*c*13	12.80 ± 0.18c	11.49 ± 0.33c	12.64 ± 0.70c	22.20 ± 1.29a	21.30 ± 2.34a	16.63 ± 0.67b	17.49 ± 2.71b	18.50 ± 2.29b
*c*10,*t*12/*t*10,*c*12	37.86 ± 0.72a	16.94 ± 2.38c	18.96 ± 1.92c	21.91 ± 0.58c	18.69 ± 2.44c	27.94 ± 0.85b	17.87 ± 0.61c	14.69 ± 3.47c
*c*9,*t*11/*t*9,*c*11	31.77 ± 0.28c	38.28 ± 2.56ab	42.92 ± 3.62a	31.57 ± 1.04c	29.77 ± 1.68c	32.19 ± 1.06bc	34.77 ± 4.19bc	36.75 ± 2.61bc
*c*8,*t*10/*t*8,*c*10	9.57 ± 0.11b	9.16 ± 0.63b	8.71 ± 0.88b	9.08 ± 1.55b	8.86 ± 0.73b	12.37 ± 0.23a	10.36 ± 2.06ab	7.31 ± 2.36b
total *c*,*t*-CLA	92.00 ± 0.65a	75.87 ± 4.79c	83.23 ± 3.71c	84.76 ± 1.68c	78.61 ± 2.32c	89.13 ± 0.88b	80.48 ± 5.18c	77.26 ± 2.35c
*c*11,*c*13	0.67 ± 0.10b	1.66 ± 0.36a	1.95 ± 0.57a	1.32 ± 0.37ab	1.16 ± 0.05b	1.03 ± 0.72ab	1.41 ± 0.40ab	0.56 ± 0.28c
*c*10,*c*12	1.51 ± 0.24b	1.58 ± 0.34ab	1.92 ± 0.94a	1.36 ± 0.23ab	2.06 ± 0.34a	1.55 ± 0.48ab	2.39 ± 0.42a	1.56 ± 0.63ab
*c*9,*c*11	1.28 ± 0.16b	2.44 ± 0.13a	2.12 ± 1.07ab	0.98 ± 0.64ab	0.69 ± 0.24b	1.56 ± 0.67ab	0.72 ± 0.34b	1.32 ± 0.90ab
*c*8,*c*10	0.35 ± 0.08b	0.45 ± 0.22ab	0.81 ± 0.58ab	0.33 ± 0.16b	0.66 ± 0.32ab	0.63 ± 0.22ab	0.51 ± 0.26ab	1.28 ± 0.90a
total *c*,*c*-CLA	3.81 ± 0.44b	6.12 ± 0.58a	6.79 ± 2.07ab	3.99 ± 0.97b	4.58 ± 0.43b	4.76 ± 1.43b	5.03 ± 0.99b	4.72 ± 1.39b

[a] Data are expressed as percent of total CLA. Values are means ± SD, $n = 5–10$. Means with different letters within a row differ significantly, $p < 0.05$.

Table 6. Relative Composition of Conjugated Linoleic Acids in Tissue Phospholipids of Hens Fed the CLA-Supplemented Diets[a]

CLA isomer	diet	serum	liver	heart	kidney	adipose	leg	breast
*t*12,*t*14	0.17 ± 0.07c	0.58 ± 0.20b	0.70 ± 0.19b	0.57 ± 0.28b	1.14 ± 0.29a	0.54 ± 0.21b	1.09 ± 0.77ab	0.36 ± 0.26bc
*t*11,*t*13	0.38 ± 0.19c	1.77 ± 0.44b	1.27 ± 0.27b	3.84 ± 0.67ab	5.31 ± 2.79ab	1.26 ± 0.06b	6.10 ± 2.03a	3.17 ± 0.74ab
*t*10,*t*12	1.27 ± 0.30b	2.54 ± 1.18ab	1.53 ± 0.42b	1.93 ± 0.22b	2.83 ± 0.81a	3.01 ± 0.38a	2.76 ± 0.86a	1.84 ± 1.05ab
*t*9,*t*11	1.35 ± 0.28c	4.71 ± 0.58ab	4.81 ± 1.25ab	3.62 ± 0.83b	7.62 ± 3.05a	4.15 ± 1.85ab	5.27 ± 2.49ab	6.02 ± 1.68a
*t*8,*t*10	0.45 ± 0.26b	0.91 ± 0.46ab	1.24 ± 0.35ab	0.42 ± 0.27b	1.32 ± 0.22ab	1.70 ± 1.02a	0.51 ± 0.19b	0.21 ± 0.11b
*t*7,*t*9	0.58 ± 0.34b	0.83 ± 0.32ab	1.07 ± 0.71ab	0.46 ± 0.21b	1.83 ± 0.37a	1.71 ± 0.96a	0.43 ± 0.20b	0.19 ± 0.02b
total *t*,*t*-CLA	4.19 ± 0.34c	11.36 ± 2.53b	10.59 ± 1.71b	10.75 ± 3.00ab	20.04 ± 7.03a	12.36 ± 4.00ab	16.16 ± 3.66ab	11.80 ± 3.02ab
*c*11,*t*13/*t*11,*c*13	12.80 ± 0.18c	7.17 ± 0.63d	8.44 ± 0.83d	28.21 ± 2.15a	24.92 ± 3.55a	15.17 ± 1.70b	23.70 ± 4.19a	21.46 ± 2.42a
*c*10,*t*12/*t*10,*c*12	37.86 ± 0.72a	21.11 ± 1.15c	21.11 ± 2.18c	19.76 ± 2.40c	16.21 ± 1.25d	28.30 ± 2.65b	12.02 ± 2.92e	8.88 ± 0.51f
*c*9,*t*11/*t*9,*c*11	31.77 ± 0.28c	47.08 ± 2.07ab	50.63 ± 1.97a	31.25 ± 1.99c	28.21 ± 4.40c	28.73 ± 1.87c	37.73 ± 7.86c	45.04 ± 1.06b
*c*8,*t*10/*t*8,*c*10	9.57 ± 0.11a	4.50 ± 0.28bc	6.85 ± 2.85b	3.42 ± 0.27c	6.47 ± 1.12b	10.00 ± 0.57a	5.12 ± 1.85b	2.46 ± 0.25c
total *c*,*t*-CLA	92.00 ± 0.65a	79.76 ± 1.68b	87.03 ± 1.94b	83.14 ± 5.40b	75.71 ± 7.92b	82.20 ± 4.64b	78.57 ± 3.53b	77.84 ± 3.42b
*c*11,*c*13	0.67 ± 0.10b	2.09 ± 0.16a	0.53 ± 0.26b	1.14 ± 0.65b	0.85 ± 0.45b	1.03 ± 0.56b	1.13 ± 0.93b	2.23 ± 0.36a
*c*10,*c*12	1.51 ± 0.24ab	1.20 ± 0.16ab	0.60 ± 0.01b	1.01 ± 0.69ab	0.67 ± 0.48b	2.39 ± 0.56ab	1.43 ± 0.91ab	2.50 ± 1.27a
*c*9,*c*11	1.28 ± 0.16c	5.01 ± 0.92a	1.16 ± 0.95c	2.33 ± 1.84bc	1.93 ± 0.76bc	0.83 ± 0.44c	2.30 ± 0.70b	5.00 ± 1.74a
*c*8,*c*10	0.35 ± 0.08bc	0.46 ± 0.21bc	0.10 ± 0.20c	1.62 ± 0.85a	0.79 ± 0.60ab	1.19 ± 0.28ab	0.41 ± 0.22b	0.63 ± 0.23b
total *c*,*c*-CLA	3.81 ± 0.44c	8.78 ± 1.35a	2.39 ± 1.09c	6.11 ± 2.45b	4.25 ± 1.93b	5.44 ± 1.25b	5.28 ± 1.79bc	10.36 ± 2.78a

[a] Data are expressed as percent of total CLA. Values are means ± SD, $n = 5–10$. Means with different letters within a row differ significantly, $p < 0.05$.

transferred to the milk lipids (*35*). Similar results were observed in other supplementation studies using different animal models (*7*, *32*−*34*). It is evident that incorporation of CLA in the tissue lipids increases as dietary CLA increases.

The present study clearly showed that incorporation of *trans/trans*, *cis/trans*, and *cis/cis* CLA isomers into the tissues of hens was a selective process. The *trans/trans* CLA isomers appeared to be preferentially incorporated into the tissue lipids, whereas the incorporation of *cis/trans* CLA isomers was partially discriminated (**Table 5**), from the observation that total *trans/trans* isomers accounted for 4.19% of total CLA in dietary lipids, but in all tissue lipids except abdominal fat and brain they reached >9.98% of total CLA isomers. In contrast, total *cis/trans* isomers were 92.00% in the diet lipids but were reduced to <84.76% in the tissue lipids (**Table 5**; **Figure 1**). Incorporation of individual CLA isomers within each group was also selective. Within the two most abundant isomers, *c*-9,*t*-11/*t*-9,*c*-11−18:2 and *c*-10,*t*-12/*t*-10,*c*-12−18:2, the former was preferentially incorporated into the tissue lipids, whereas the latter did not accumulate proportionally to its relative abundance in the diet (**Table 5**; **Figure 1**). Among the *trans/trans* group, *t*-11,*t*-13−18:2, *t*-10,*t*-12−18:2, and *t*-9,*t*-11−18:2 incorporated into the tissue lipids in proportions greater than their abundance in the diet (**Table 5**; **Figure 1**). These observations are consistent with our previous reports in egg yolk (*36*) and in the liver of rat neonates (*35*). We are unaware of any studies examining the isomeric distribution in tissues in relation to that in the diet except for that of Kramer et al. (*34*), who studied the distribution of CLA isomers in various tissue lipid classes of pigs fed a CLA diet using both GLC and Ag-HPLC. They found a preferential incorporation of *c*-9,*t*-11−18:2 into the liver PL. This is consistent with the present observation that *c*-9,*t*-11/t-9,c-11−18:2 was selectively accumulated into the liver and serum PL but not in heart and kidney. The reverse is seen for

Table 7. Relative Composition of Conjugated Linoleic Acids in Tissue Triacylglycerols of Hens Fed the CLA-Supplemented Diets[a]

CLA isomer	diet	serum	liver	heart	kidney	adipose	leg	breast
*t*12,*t*14	0.17 ± 0.07c	0.32 ± 0.18bc	0.59 ± 0.32bc	0.61 ± 0.14b	0.86 ± 0.27cab	1.47 ± 0.62a	0.56 ± 0.27b	0.55 ± 0.25b
*t*11,*t*13	0.38 ± 0.19d	1.21 ± 0.40cd	2.42 ± 1.40ab	1.00 ± 0.48c	2.12 ± 0.81b	1.94 ± 0.96bc	2.31 ± 1.62ab	3.76 ± 0.41a
*t*10,*t*12	1.27 ± 0.30c	2.80 ± 1.25ab	3.67 ± 1.89b	2.87 ± 0.76b	2.42 ± 0.28b	2.26 ± 0.12b	2.44 ± 0.84b	5.22 ± 1.60a
*t*9,*t*11	1.35 ± 0.28c	3.70 ± 0.94ab	3.39 ± 1.57ab	2.75 ± 0.63b	1.60 ± 0.73bc	2.29 ± 0.42b	3.20 ± 0.97ab	4.32 ± 0.44a
*t*8,*t*10	0.45 ± 0.26b	1.37 ± 0.62b	2.05 ± 0.81ab	1.39 ± 0.68b	0.71 ± 0.15b	1.52 ± 0.81ab	0.72 ± 0.27b	2.37 ± 0.35a
*t*7,*t*9	0.58 ± 0.34c	1.46 ± 0.36b	2.03 ± 0.75a	2.06 ± 0.74ab	1.89 ± 0.71b	1.92 ± 0.18b	0.66 ± 0.29c	2.44 ± 0.33a
total *t*,*t*-CLA	4.19 ± 0.34c	10.87 ± 3.46b	14.16 ± 5.8ab	10.67 ± 1.16b	9.60 ± 1.05b	11.40 ± 2.59b	9.89 ± 2.89b	18.67 ± 1.83a
*c*11,*t*13/*t*11,*c*13	12.80 ± 0.18b	15.61 ± 1.23ab	15.43 ± 1.39ab	15.62 ± 2.29ab	17.20 ± 1.29ab	16.15 ± 1.62ab	19.19 ± 2.89a	15.44 ± 1.76ab
*c*10,*t*12/*t*10,*c*12	37.86 ± 0.72a	12.64 ± 1.16c	13.98 ± 0.71c	24.51 ± 1.30b	25.59 ± 0.45b	23.00 ± 4.24b	21.72 ± 1.69b	19.20 ± 1.73b
*c*9,*t*11/*t*9,*c*11	31.77 ± 0.28b	37.94 ± 2.47a	37.38 ± 3.75a	29.68 ± 0.92b	30.90 ± 0.99b	26.66 ± 2.90b	32.24 ± 1.49b	26.77 ± 2.74b
*c*8,*t*10/*t*8,*c*10	9.57 ± 0.11b	12.36 ± 1.87a	11.61 ± 2.73ab	15.25 ± 1.16a	11.35 ± 1.58ab	14.89 ± 5.75ab	12.32 ± 3.36ab	10.56 ± 2.64ab
total *c*,*t*-CLA	92.00 ± 0.65a	78.54 ± 5.79c	78.40 ± 8.00bc	85.07 ± 1.61b	85.03 ± 1.73b	80.70 ± 3.30bc	85.47 ± 2.73b	73.96 ± 3.49c
*c*11,*c*13	0.67 ± 0.10b	2.48 ± 1.07a	1.78 ± 0.37a	1.49 ± 0.53a	1.59 ± 0.28a	1.20 ± 0.30a	1.68 ± 0.42a	1.74 ± 0.52a
*c*10,*c*12	1.51 ± 0.24b	1.71 ± 0.87ab	1.70 ± 0.63ab	1.35 ± 0.29b	1.43 ± 0.81ab	1.56 ± 0.16b	0.97 ± 0.35b	2.84 ± 1.04a
*c*9,*c*11	1.28 ± 0.16b	6.40 ± 2.80ab	2.60 ± 1.39b	1.05 ± 0.76b	1.89 ± 0.97b	4.54 ± 0.50a	0.99 ± 0.39b	1.65 ± 0.25b
*c*8,*c*10	0.35 ± 0.08b	ND	1.36 ± 0.61a	0.36 ± 0.05b	0.46 ± 0.18b	0.60 ± 0.16ab	1.00 ± 0.46a	1.13 ± 0.92a
total *c*,*c*-CLA	3.81 ± 0.44b	10.59 ± 4.58ab	7.45 ± 2.30a	4.26 ± 1.22ab	5.37 ± 1.73ab	7.90 ± 1.06ab	4.64 ± 0.42ab	7.36 ± 1.73a

[a] Data are expressed as percent of total CLA. Values are means ± SD, $n = 5–10$. Means with different letters within a row differ significantly, $p < 0.05$.

c-10,*t*-12/*t*-10,*c*-12−18:2, which was consistently incorporated into all tissue PL and TG in proportions less than that in the diet (**Table 6**). Serum and liver TG preferentially accumulated *c*-9,*t*-11/*t*-9,*c*-11−18:2, whereas other tissue TG incorporated this isomer in an unselective manner. All observations support the view that isomeric distributions of CLA isomers vary with not only the tissues but also the lipid classes.

The mechanism of selective deposition of CLA isomers into the tissues of hens remains unknown. It is unlikely due to selective absorption. This rationalization is based on the following observations: (i) no difference in isomeric distribution was found between the adipose tissue of pig and the diet (*34*) and (ii) CLA isomeric composition in the milk was similar to that in the maternal diet (*35*). Compared with the *cis/trans* group, the *tran/trans* group was selectively incorporated in the tissue lipids. It is most likely that the accumulation of *trans/trans* CLA isomers was the result of slower metabolism, poor substrates for oxidation, and preferred geometrical insertion in the tissue lipids of hens.

The impact of CLA supplementation on the other fatty acid composition was also noted, with 22:6*n*−3 being decreased in all tissues except brain. First, the difference in fatty acid composition between the control and CLA group would partially reflect that in diet used to feed hens. Second, it is likely that *cis/trans* CLA isomers compete with 18:3*n*−3 for desaturases and elongase, leading to a decreased level of 22:6*n*−3. It is interesting that the impact of CLA supplementation on the egg yolk fatty acid composition was more pronounced than that on tissue lipids. Our previous report showed that supplementation of CLA in the diet of laying hens not only decreased the contents of 18:1*n*−9, 20:4*n*−6, and 22:6*n*−3 but also increased those of 18:0, 18:3*n*−3, and 16:0 (*36*). The present result showed that supplementation of CLA led to an increase in 18:0 but to a decrease in 18:1*n*−9 in the liver lipids (**Table 3**), which is in agreement with the results of Chamruspollert and Sell (*31*).

CLA is a mixture of more than 12 isomers. No data available to date have studied and compared the biological activities of each isomer. It is suggested that *c*-9,*t*-11−18:2 is the active isomer (*10*, *27*), but several recent papers claim that *t*-10,*c*-12−18:2 is also biologically potent (*28*, *29*). Together with the previous studies (*31*−*33*, *36*), the present results show that the transfer of CLA isomers from the diet to the tissue and egg yolk lipids was efficient. Although *cis/trans* CLA isomers were the major isomers in diet and tissues, the transfer process was selective, with the *trans/trans* isomers being preferentially accumulated and *cis/trans* isomers being partially discriminated relative to the amounts in the diet. Regarding *c*-9,*t*-11/*t*-9,*c*-11−18:2 and *c*-10,*t*-12/*t*-10,*c*-12−18:2, the two most abundant isomers, the incorporation of the former into the tissue lipids was more preferred than that of the latter. Once the biological potency of each isomer is known, it will be ideal to synthesize a most potent CLA isomer as an animal feed to enrich eggs and meats.

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