Maternal Dietary Conjugated Linoleic Acid Alters Hepatic Triacylglycerol and Tissue Fatty Acids in Hatched Chicks

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ABSTRACT: The effects of feeding CLA to hens on newly hatched chick hepatic and carcass lipid content, liver TAG accumulation, and FA incorporation in chick tissues such as liver, heart, brain, and adipose were studied. These tissues were selected owing to their respective roles in lipid assimilation (liver), as a major oxidation site (heart), as a site enriched with long-chain polyunsaturates for function (brain), and as a storage depot (adipose). Eggs with no, low, or high levels of CLA were produced by feeding hens a corn–soybean meal–basal diet containing 3% (w/w) corn oil (Control), 2.5% corn oil + 0.5% CLA oil (CLA1), or 2% corn oil + 1.0% CLA oil (CLA2). The egg yolk content of total CLA was 0.0, 1.0, and 2.6% for Control, CLA1, and CLA2, respectively ($P < 0.05$). Maternal dietary CLA resulted in a decrease in chick carcass total fat ($P < 0.05$). Liver tissue of CLA2 chicks had the lowest fat content ($P < 0.05$). The liver TAG content was 8.2, 5.8, and 5.1 mg/g for Control, CLA1, and CLA2 chicks, respectively ($P < 0.05$). The chicks hatched from CLA1 and CLA2 incorporated higher levels of *cis*-9,*trans*-11 CLA in the liver, plasma, adipose, and brain than Control ($P < 0.05$). The content of 18:0 was higher in the liver, plasma, adipose, and brain of CLA1 and CLA2 than Control ($P < 0.05$), but no difference was observed in the 18:0 content of heart tissue. A significant reduction in 18:1 was observed in the liver, plasma, adipose, heart, and brain of CLA1 and CLA2 chicks ($P < 0.05$). DHA (22:6n-3) was reduced in the heart and brain of CLA1 and CLA2 chicks ($P < 0.05$). No difference was observed in carcass weight, dry matter, or ash content of chicks ($P > 0.05$). The hatchabilities of fertile eggs were 78, 34, and 38% for Control, CLA1, and CLA2, respectively ($P < 0.05$). The early dead chicks were higher in CLA1 and CLA2 than Control (18 and 32% compared with 9% for Control), and alive but not hatched chicks were 15 and 19% for CLA1 and CLA2, compared with 8% for Control ($P < 0.05$). Maternal supplementation with CLA leads to a reduction in hatchability, liver TAG, and carcass total fat in newly hatched chicks.

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CLA have received considerable attention for their potential to repartition body mass in growing animals (1–3) and for their suggested health-promoting effects (4–6). CLA is a collective term used for a group of positional and geometric dienoic isomers of essential linoleic acid (18:2n-6). Among the CLA isomers, *cis*-9,*trans*-11 (*c*9,*t*11) and *trans*-10,*cis*-12 (*t*10,*c*12) are the predominant ones found in foods of ruminant origins where they are synthesized by rumen microbes. Because a major proportion of fat in the U.S. diet is of animal origin, feeding strategies have been adopted to increase the content of CLA in foods of ruminant as well as nonruminant origin, such as pork, chicken eggs, and meat (7–9), because of the possible health-promoting properties of CLA (4–6).

Enriching eggs with up to 14.8% CLA has been reported through manipulation of the diets of laying hen (10). CLA incorporation in eggs resulted in a significant increase in saturated FA (SFA) (16:0, 18:0) with a concomitant reduction in monounsaturated FA(MUFA) (16:1, 18:1n-9) (10,11). However, feeding CLA had little effect on the total fat content of egg. Yolk fat is primarily TAG (68%) and phospholipids (28%). During the 21-d incubation period, the chicken embryo utilizes over 80% of the yolk fat for energy production, for formation of fat stores, and for synthesis of membrane phospholipids (12,13). The yolk-derived lipids are reassembled into TAG-rich lipoproteins by the endodermal cells of the yolk sac membrane (12). Tissue maturation in the embryo is also dependent on the incorporation of highly characteristic FA profiles into cell membrane phospholipids. Recent studies suggest that phospholipids containing long-chain n-6 and n-3 PUFA are involved in many cellular signaling mechanisms such as cardiac rhythm, neurotransmission, and photoreception (14). Therefore, FA delivery, partitioning, and tissue uptake during incubation affects embryonic health and hatchability. Adverse effects of yolk CLA on hatchability (15), yolk absorption and reduction in chick VLDL TAG were reported (16). Because the CLA composition of egg yolk can be manipulated by diet, the CLA-modified egg is a useful research tool for studying the role of maternal dietary CLA on lipid metabolism in the progeny. Given that CLA are known to have significant effects on lipid partitioning in mammals, it is important to evaluate the extent to which supplementation of CLA during development through maternal supply (yolk) influences FA composition and TAG allocation in hatched chicks. The objectives of the present study were to investigate the effects of feeding CLA to hens on chick hepatic and carcass lipid content, liver TAG accumulation, and FA incorporation into chick tissues such as liver, heart, brain, and adipose. These tissues were selected because of

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Abbreviations: *c*9,t11, *cis* 9,*trans* 11 CLA; *t*10,*c*12, *trans*-10,*cis*-12; MUFA, monounsaturated FA; SCD-1, stearoyl-CoA desaturase-1; SFA, saturated FA

their respective roles in lipid assimilation (liver), oxidation (heart), functional long-chain PUFA incorporation (brain), and storage (adipose).

MATERIALS AND METHODS

These experiments were reviewed by the Oregon State University Animal Care and Use Committee to ensure adherence to Animal Care Guidelines.

Maternal diet and egg enrichment of CLA. Eggs with different levels of CLA were obtained by feeding New Hampshire breeder hens a corn–soybean meal-based diet containing 3% corn oil (Control), 2.5% corn oil + 0.5% CLA oil (CLA1), and 2% corn oil + 1% CLA oil (CLA2). All the diets were isoenergetic (2900 kcal/kg feed) and isonitrogenous (16% crude protein). The FA composition of the diets is shown in Table 1. Corn oil was purchased from a local market. The CLA oil containing 75% FFA oil was donated from a commercial source (CLA One®; Pharmanutrients, Lake Bluff, IL) and was made of approximately equal amounts of *c*9,*t*11 and *t*10,*c*12. The other FA in the CLA oil were 16:0 (4.4%), 18:0 (2.8%), 18:1 (15.4%), and 18:2 n-6 (1.8%). The diets were mixed weekly and were stored in a cold room (4°C) in airtight containers. The experimental diets were fed to hens ($n = 30$; 10 per treatment) for a period of 6 wk. The birds were kept individually in cages and were maintained on a 16:8 light/dark photoperiod and standard conditions of temperature and ventilation as per Oregon State University Poultry Farm standard operating procedures. Water and feed were provided *ad libitum.*

Sample collection. After 5 wk on experimental diet, hens were artificially inseminated with 0.05 mL of pooled semen collected immediately before insemination. Eggs were gathered for 10 d after insemination and were held in a cold room at 65°F (18.3°C). All eggs were warmed to room temperature before setting in the incubator. All the eggs were distributed randomly in the incubator. A total of 180 eggs (60 eggs per treatment) were incubated at 37°C and 85% RH. Hatching times were observed from day 21 onward in the morning and evening. The hatched chicks from all treatments were counted in the early morning of Day 22. The eggs that did not hatch were removed from the incubator and were also counted. The eggs were broken open and the number of embryos that were dead or pips (i.e., alive and pipped through the shell, but not free of the shell) were also counted. The hatched chicks (five per treatment) were sacrificed, and heart, adipose, liver, and blood (1 mL) were collected. From the remaining hatched chicks five were taken for proximate analysis. All samples were stored at −80°C and were analyzed within 2 mon of collection.

TABLE 1
Major FA Composition (%) of Maternal Diets

Dietary treatments[a]			
FA	Control	CLA1	CLA 2
14:0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
16:0	13.5 ± 0.7	13.3 ± 0.1	13.2 ± 0.1
18:0	1.9 ± 0.1	1.9 ± 0.1	1.9 ± 0.1
18:1	25.4 ± 0.8	24.0 ± 0.6	23.4 ± 0.7
18:2	56.8 ± 0.3	52.8 ± 0.1	49.1 ± 0.2
18:3n-3	2.4 ± 0.0	2.4 ± 0.1	2.2 ± 0.1
Cis-9,*trans*-11 CLA	0.0 ± 0	2.6 ± 0.0	4.7± 0.1
Trans-10,*cis*-12 CLA	0.0 ± 0	2.9 ± 0.0	5.3 ± 0.2
Total SFA	15.5 ± 0.5	15.3 ± 0.2	15.2 ± 0.3
Total MUFA	25.4 ± 0.6	23.9 ± 0.1	23.5 ± 0.2
Total CLA	0.0 ± 0.0	5.6 ± 0.1	10.0 ± 0.1
Total n-6 PUFA	56.7 ± 0.3	52.8 ± 0.1	49.1 ± 0.4
Total n-3 PUFA	2.4 ± 0.0	2.4 ± 0.1	2.2 ± 0.1

[a]Control diet contained 3% corn oil. CLA1 and CLA2 represent corn oil + 0.5% CLA or corn oil + 1% CLA, respectively. SFA, saturated FA; MUFA, monounsaturated FA.

Carcass pressure cooking. The carcasses were autoclaved individually in pyrex beakers at 121°C (15 psi) for 2.5 h. When cool, the contents of each beaker were homogenized in a 4-L Waring heavy-duty laboratory blender on high speed (20,000 rpm) for 5 min. The resulting slurry was taken for lipid and proximate analysis according to methods of the Association of Official Analytical Chemists (17).

Lipid analyses. Total lipids were extracted from feed, egg yolk, liver, heart, adipose, plasma, and carcass slurry by the method of Folch *et al.* (18). About 1 g of yolk, feed, carcass slurry, adipose, or whole tissues (heart, brain, liver) was weighed into a screw-capped test tube with 18 mL of chloroform/methanol (2:1, vol/vol), and homogenized with a polytron (Type PT10/35; Brinkman Instruments, Westbury, NY) for 15 to 20 s at high speed. After an overnight incubation at 4°C, 4 mL of 0.88% sodium chloride solution was added and mixed. The phases were separated by centrifugation, and the lower chloroform layer was collected for lipid and FA analysis. Total lipids were determined gravimetrically for liver, egg yolk, and carcass. The lipid extract (2 mL) was taken into a 16-mL screw-capped glass tube and dried in a block heater at 39°C under a gentle stream of nitrogen. The dried lipids were resolubilized in 2 mL of boron trifluoride/methanol (10% w/w) and were heated in a 95–100°C water bath for 60 min and FAME were prepared. The FAME were separated and quantified by GC.

Analysis of FA composition was performed with an HP 6890 gas chromatograph (Hewlett-Packard Co., Wilmington, DE) equipped with an autosampler, FID, and fused-silica capillary column, 100 m × 0.25 mm × 0.2 μm film thickness (SP-2560; Supelco, Bellefonte, PA). Sample (2 μL) was injected with helium as a carrier gas onto the column programmed for ramped oven temperatures (initial temperature was 110°C, held for 0.5 min, then ramped at 20°C/min to 200°C and held for 50 min, then ramped at 10°C/min to 230°C and held for 5.0 min). Inlet and detector temperatures were both 250°C (12). Peak areas and percentages were calculated using Hewlett-Packard ChemStation software. FAME were identified by comparison with retention times of authentic standards (Matreya, Pleasant Gap, PA). FA values and total lipids are expressed as weight percentages.

Liver TAG were estimated by adapting an enzyme-based procedure with a colorimetric end point, originally developed for serum as reported earlier (19). The liver tissue total lipid extract served as substrate. A glycerol standard (G1394-5ML;

Sigma Chemical, St. Louis, MO) was used for calibration of the assay, and Accutrol normal control serum (A2034-1VL; Sigma Chemical) for quality control.

Statistical analysis. The effects of maternal diet on carcass lipids, hepatic and plasma lipids, TAG, and FA were analyzed by ANOVA using SAS (version 8.2) (SAS Institute, Cary, NC) (20). Student–Newman–Keuls multiple range test (21) was used to compare differences among treatment means ($P < 0.05$). Mean values and SEM are reported.

RESULTS AND DISCUSSION

All the diets were isoenergetic, isonitrogenous, and had added 3% oil, which was within the limits of energy and protein supplied to breeder hens. Addition of CLA oil altered the *c*9,*t*11 and *t*10,*c*12 content of the diets (Table 1). CLA were present only in the CLA-supplemented diets (CLA1 and CLA2) and consisted of both *c*9,*t*11 and *t*10,*c*12. Incorporating CLA oil in the diet resulted in a significant increase in *c*9,*t*11 and *t*10,*c*12 content of eggs (Fig. 1A). The content of total CLA was 1.0 and 2.6%, respectively, in CLA1 and CLA2 eggs. Inclusion of CLA also resulted in an increase in SFA (16:0, 18:0) with a concomitant reduction in MUFA (18:1) resulting in an increase in SFA/MUFA ratio in CLA1 and CLA2 eggs (Fig. 1B). These results also corroborate our previous reported results and those of others (10,11). This decrease in MUFA may be due to inhibition of the expression of the stearoyl-CoA desaturase-1 (SCD-1) enzyme that converts 16:0 and 18:0 to 16:1 and 18:1 by CLA, respectively (22). Inclusion of CLA did not alter the total lipid content of egg, which was 30.6, 31.2, and 29.9 for Control, CLA1, and CLA2, eggs, respectively ($P > 0.05$).

Liver lipids, TAG, and FA. A significant effect of dietary CLA on hepatic total lipid was noted. Livers of chicks hatched from hens fed CLA2 were lower in total lipids than Control chicks ($P < 0.05$) (Fig. 2A). The decrease in liver lipids by yolk CLA could be the consequence of a higher oxidation rate of these FA as reported in mice fed *t*10,*c*12 CLA (23). The decrease in liver lipids was associated with a decrease in hepatic TAG concentration in both CLA1 and CLA2 chicks ($P < 0.05$) (Table 2). To our knowledge, effects of maternal dietary CLA on liver TAG concentrations in hatched chicks have not been previously reported. Transfer of lipids and fat-soluble nutrients from the egg yolk to the embryonic liver is accomplished by TAG-rich lipoproteins through the yolk sac membrane (24). The percentage of TAG in VLDL particles has been reported to be lower in chicks hatched from hens fed CLA (16). These researchers also observed a reduction in remnant yolk in the chicks hatched from hens fed CLA, suggesting that CLA enrichment in the yolk leads to either an impairment in yolk lipid removal or hepatic tissue TAG accretion during the incubation period. Since yolk TAG is the main source of energy for avian embryos, a lack of mobilization or an impairment in absorption may affect chick health. In our study, the percentages of early dead chicks were higher in CLA1 and CLA2 than Control (18 and 32% compared with 9% for Control), and alive but not hatched (pipped) chicks were 15 and 19% for CLA1 and CLA2 compared with 8% for Control. The hatchabilities of fertile eggs were 78, 34, and 38% for Control, CLA1, and CLA2, respectively ($P < 0.05$). CLA have been reported to accumulate in the liver mitochondrial matrix affecting the oxidation of other FA (23). Because the avian embryo derives 90% of its energy from oxidation of FA, a reduced energy source may lead to embryonic death or an increase in live but not hatched chicks, as noted in our study.

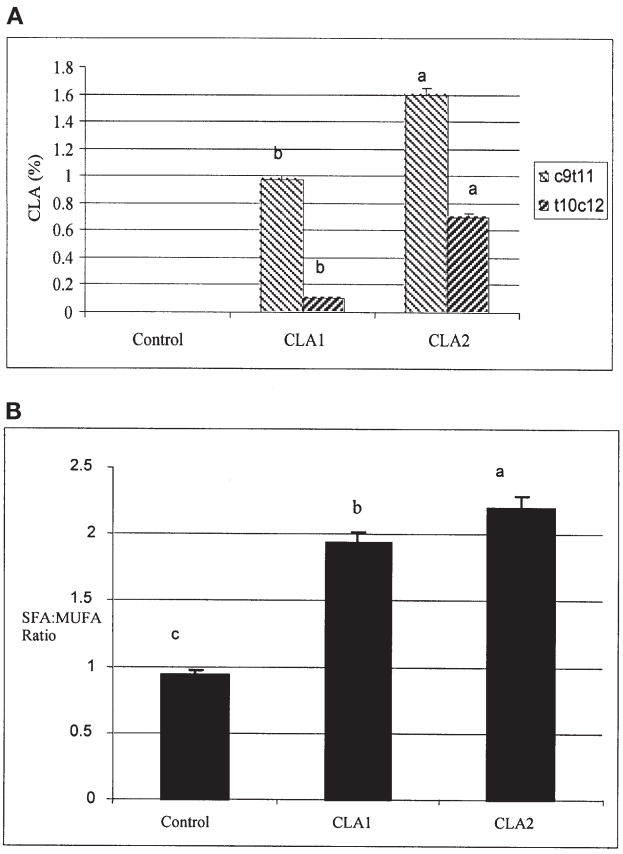

FIG. 1. CLA isomers (%) (A) and total saturated to monounsaturated ratio (B) in the egg yolk from breeder hens fed diets containing 0.0, 0.5%, or 1.0% CLA. [a–c]Means with different superscripts differ significantly for each bar ($P < 0.05$) ($n = 5$). Control diet contained 3% corn oil. CLA1 and CLA2 represent 2.5% corn oil + 0.5% CLA or 2% corn oil +1% CLA.

An increase in egg yolk CLA resulted in a dramatic change in the chick liver SFA and MUFA content (Table 2). A significant increase in 18:0 with a concomitant decrease in 18:1 was observed in CLA1 and CLA2 chicks. The inhibitory action of CLA on SCD-1, the enzyme capable of converting 18:0 to 18:1, has been reported (22). In the present study, yolk and liver 18:0 was significantly higher in the CLA1 and CLA2 groups, indicating the inhibitory action of maternal CLA on SCD-1. An alteration of Δ-6 desaturase in chick liver due to an altered n-6/n-3 FA ratio in the maternal diet has been reported (25). These results suggest that maternal FA may have a profound effect on liver enzyme activities affecting unsaturated FA metabolism during avian embryonic development. Total CLA

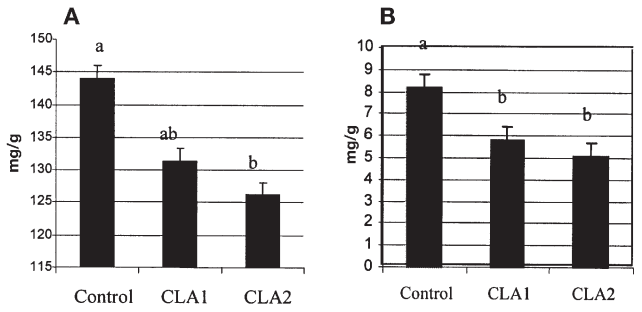

FIG. 2. Hepatic total lipid (A) and TAG (B) content of newly hatched chicks from breeder hens fed diets with or without CLA. [a–b]Means with different superscripts differ significantly ($P < 0.05$) ($n = 5$). Control diet contained 3% corn oil. CLA1 and CLA2 represent 2.5% corn oil + 0.5% CLA or 2% corn oil +1% CLA.

were higher in the liver of CLA2 chicks. The *c*9,*t*11 isomer was the predominant isomer and was higher ($P < 0.05$) in livers from CLA1 and CLA2 chicks (Table 2). In addition to the changes in SFA and MUFA, the content of 18:2n-6 and total n-6 FA was higher in CLA1 and CLA2 chicks ($P < 0.05$). The level of DHA (22:6n-3) was lower in CLA2 chicks ($P < 0.05$).

The FA composition of chick plasma, adipose, heart, and brain tissue is shown in Tables 3 and 4. Stearic acid (18:0) was increased significantly in the plasma and brain of CLA1 and CLA2 chicks compared with the Control (Table 3). However, no difference was observed in the heart tissue content of 18:0 in CLA1 and CLA2, suggesting that the activities of SCD-1 in chickens may be tissue-specific. Consistent with findings in the liver, 18:1 was significantly reduced in the plasma, heart, and brain tissue of CLA1 and CLA2 chicks (Tables 3 and 4). Inclusion of CLA also resulted in a significant increase in linoleic acid (18:2 n-6) in CLA1 and CLA2 chicks in the plasma, heart, and brain. The enrichment of total CLA due to maternal diet in the order of magnitude was adipose > liver > plasma > brain. No CLA was detected in the heart tissue. The contents of long-chain n-6 PUFA such as arachidonic acid (20:4n-6), 22:4n-6, and 22:5n-6 were higher in the brain tissue of CLA1 and CLA2 chicks than in Control chicks ($P < 0.05$). However, DHA (22:6n-3) was lower in the heart and brain tissue of CLA1 and CLA2 chicks by maternal supplementation of CLA than in control chicks ($P < 0.05$). Although an adverse effect of yolk CLA on hatchability has been reported (15), very few studies have investigated the role of egg CLA during avian embryonic development. The alteration of n-6 and n-3 FA observed in the chick tissues in the current study may also affect eicosanoid metabolism in these tissues. Feeding CLA has been reported to reduce the levels of brain prostaglandin E_2 in mice (26). The decrease in the concentration of 18:1 and the altered SFA/MUFA ratio, along with impaired eicosanoid metabolism

TABLE 2
Major FA Composition (%) of Newly Hatched Chick Liver Total Lipids

FA	Dietary treatments[a]		
	Control	CLA1	CLA2
14:0	0.60 ± 0.0[b]	0.91 ± 0.0[a]	0.99 ± 0.0[a]
16:0	10.2 ± 0.8	9.8 ± 0.8	9.6 ± 0.9
16:1	1.0 ± 0.3	0.8 ± 0.1	0.8 ± 0.1
18:0	11.1 ± 1.0[b]	14.9 ±1.0[a]	15.3 ± 1.2[a]
18:1 n-9	49.1 ± 3.6[a]	36.5 ± 2.0[b]	33.7 ± 3.1[b]
18:2 n-6	16.4 ± 0.7[c]	24.1 ± 1.2[b]	25.8 ± 1.2[a]
18:3 n-3	0.0 ± 0.0[c]	0.5 ± 0.0[b]	0.6 ± 0.0[a]
20:4 n-6	8.0 ± 1.0	8.3 ± 0.4	7.8 ± 1.1
Cis-9,*trans*-11 CLA	0.0 ± 0.0[c]	0.7 ± 0.1[b]	1.6 ± 0.1[a]
Trans-10,*cis*-12 CLA	0.0 ± 0.0[b]	0.0 ± 0.0[b]	0.3 ± 0.2[a]
22:4 n-6	0.1 ± 0.2	0.4 ± 0.2	0.4 ± 0.2
22:5 n-6	1.2 ± 0.2	1.1 ± 0.1	1.0 ± 0.2
22:6 n-3	2.3 ± 0.4[a]	1.8 ± 0.3[ab]	1.4 ± 0.4[b]
Total SFA	21.2 ±1.4[b]	24.7 ± 1.6[a]	25.2 ± 2.1[a]
Total MUFA	50.5 ± 3.5[a]	38.1 ± 2.0[b]	35.5 ± 2.8[b]
Total n-6 PUFA	25.9 ± 2.0[b]	34.1 ± 1.3[a]	35.3 ± 0.7[a]
Total n-3 PUFA	2.3 ± 0.4	2.3 ± 0.3	2.2 ± 0.4

[a]Control diet contained 3% corn oil. CLA1 and CLA2 represent 2.5% corn oil + 0.5% CLA or 2% corn oil +1% CLA. [a-c]Means ± SD with different superscripts within a row differ significantly ($P < 0.05$) ($n = 5$). For abbreviations see Table 1.

TABLE 3
Major FA Composition (%) of Newly Hatched Chick Plasma and Adipose Tissue[a]

FA	Dietary treatments		
	Control	CLA1	CLA2
	Plasma		
14:0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
16:0	19.7 ± 1.2	20.2 ± 1.3	21.1 ± 1.1
16:1	0.9 ± 0.5	1.0 ± 0.2	0.8 ± 0.2
18:0	11.9 ± 0.8[b]	14.5 ± 0.4[a]	15.1 ± 0.9[a]
18:1 n-9	30.1 ± 1.4[a]	20.8 ± 1.3[b]	18.6 ± 1.5[c]
18:2 n-6	25.4 ± 0.9[b]	31.1 ± 1.8[a]	33.8 ± 1.1[a]
20:4 n-6	9.3 ± 1.0[a]	9.3 ± 1.3[a]	6.8 ± 1.7[b]
Cis-9,*trans*-11 CLA	0.0 ± 0.0[b]	0.5 ± 0.1[a]	0.7 ± 0.1[a]
Trans-10,*cis*-12 CLA	0.0 ± 0	0.0 ± 0	0.1 ± 0.0
22:5 n-6	0.6 ± 0.1	0.8 ± 0.1	0.6 ± 0.2
22:6 n-3	0.6 ± 0.3	0.6 ± 0.2	0.2 ± 0.0
Total SFA	32.2 ± 1.1[c]	34.9 ± 1.7[ab]	36.7 ± 0.2[a]
Total MUFA	31.9 ± 2.4[a]	22.4 ± 1.1[b]	20.4 ± 2.1[b]
Total n-6 PUFA	35.4 ±1.3[b]	41.2 ± 1.6[a]	41.4 ± 0.9[a]
Total n-3 PUFA	0.6 ± 0.1[b]	1.4 ± 0.1[a]	0.9 ± 0.1[a]
Total CLA	0.0 ± 0[c]	0.5 ± 0.1[b]	0.8 ± 0.1[a]
	Adipose		
14:0	0.8 ± 0.0[b]	0.9 ± 0.1[ab]	0.9 ± 0.1[a]
16:0	28.9 ± 0.3[c]	31.8 ± 0.8[b]	32.7 ± 0.6[a]
16:1	2.1 ± 0.2[a]	1.0 ± 0.2[b]	0.3 ± 0.3[c]
18:0	7.0 ± 0.6[c]	9.4 ± 0.7[b]	10.8 ± 0.3[a]
18:1 n-9	35.2 ± 1.6[a]	21.4 ± 0.6[b]	18.8 ± 0.4[c]
18:2 n-6	24.0 ± 1.2[b]	33.1 ± 0.8[a]	32.5 ± 0.6[a]
18:3 n-3	0.3 ± 0.2[b]	0.9 ± 0.1[a]	1.0 ± 0.1[a]
20:4 n-6	1.5 ± 0.2[a]	0.9 ± 0.1[b]	0.6 ± 0.1[b]
Cis-9,*trans*-11 CLA	0.0 ± 0[c]	0.7 ± 0[b]	1.4 ± 0[a]
Trans-10,*cis*12 CLA	0.0 ± 0[c]	0.3 ± 0[b]	0.8 ± 0.1[a]
Total SFA	36.7 ± 0.6[c]	42.1 ± 1.4[b]	44.5 ± 1.0[a]
Total MUFA	37.4 ± 1.8[a]	22.3 ± 0.6[b]	19.1 ± 0.6[c]
Total n-6 PUFA	25.5 ± 1.4[b]	34.0 ± 0.9[a]	33.1 ± 0.7[a]
Total n-3 PUFA	0.4 ± 0[b]	0.9 ± 0[a]	0.9 ± 0[a]

[a]Control diet contained 3% corn oil. CLA1 and CLA2 represent 2.5% corn oil + 0.5% CLA or 2% corn oil + 1% CLA. [a-c]Means ± SD with different superscripts within a row differ significantly ($P < 0.05$) (n = 5). For abbreviations see Table 1.

TABLE 4
Major FA Composition (%) of Newly Hatched Chick Heart and Brain Tissue[a]

FA	Dietary treatments		
	Control	CLA1	CLA2
		Heart	
14:0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
16:0	20.9 ± 0.9	21.5 ± 1.0	22.7 ± 0.9
16:1	2.4 ± 0.2	3.1 ± 0.2	2.9 ± 0.8
18:0	20.6 ± 1.1	19.8 ± 0.9	21.1 ± 1.1
18:1 n-9	14.1 ± 1.3[a]	11.0 ± 1.1[b]	9.9 ± 0.4[b]
18:2 n-6	12.3 ± 0.8[b]	19.1 ± 1.2[a]	17.3 ± 0.9[a]
20:2 n-6	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
20:4 n-6	21.2 ± 0.5	19.7 ± 0.9	20.3 ± 0.8
22:4 n-6	0.1 ± 0.0[a]	0.0 ± 0.0[b]	0.0 ± 0.0[b]
22:5 n-6	1.5 ± 0.5	1.3 ± 0.2	1.2 ± 0.3
22:6 n-3	2.5 ± 0.1[a]	1.6 ± 0.2[b]	1.5 ± 0.2[b]
Total SFA	43.3 ± 1.5	43.0 ± 1.2	45.6 ± 1.4
Total MUFA	17.0 ± 1.9[a]	14.1 ± 0.6[b]	12.9 ± 1.2[b]
Total n-6 PUFA	37.2 ± 0.6	41.3 ± 0.6	39.8 ± 0.9
Total n-3 PUFA	2.5 ± 0.1[a]	1.6 ± 0.4[b]	1.7 ± 0.2[b]
		Brain	
14:0	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0
16:0DMA	3.2 ± 0.1	3.3 ± 0.1	3.2 ± 0.9
16:0	30.4 ± 0.5	30.4 ± 0.8	29.7 ± 3.2
16:1	4.4 ± 0.2[b]	4.6 ± 0.3[b]	5.1 ± 1.4[a]
18:0	16.9 ± 0.4[b]	17.9 ± 0.2[a]	18.3 ± 2.5[a]
18:1 n-9	14.3 ± 0.4[a]	11.6 ± 0.4[b]	10.5 ± 0.3[c]
18:2 n-6	2.1 ± 0.1[c]	3.1 ± 0.2[b]	3.7 ± 0.4[a]
20:1n-9	0.4 ± 0.0[a]	0.2 ± 0.2[b]	0.2 ± 0.1[b]
20:2 n-6	0.5 ± 0.1[c]	0.9 ± 0.1[b]	1.1 ± 1.3[a]
20:3 n-6	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.1
20:4 n-6	9.8 ± 0.2[b]	10.6 ± 0.4[a]	10.6 ± 1.5[a]
20:5 n-3	0.0 ± 0.1[b]	0.0 ± 0.1[b]	0.3 ± 0.1[a]
22:0	0.2 ± 0[ab]	0.1 ± 0[b]	0.3 ± 0.1[a]
22:4 n-6	2.0 ± 0.1[c]	2.2 ± 0.1[b]	2.4 ± 0.2[a]
Cis-9,*trans*-11 CLA	0.0 ± 0.0[b]	0.1 ± 0.1[b]	0.5 ± 0.2[a]
22:5 n-6	2.9 ± 0.4[b]	3.6 ± 0.2[a]	3.6 ± 0.5[a]
22:5 n-3	0.1 ± 0.1[c]	0.4 ± 0.0[b]	0.6 ± 0.1[a]
22:6 n-3	10.6 ± 0.4[a]	9.5 ± 0.3[b]	8.6 ± 0.6[b]
24:1	0.0 ± 0	0.3 ± 0.1	0.2 ± 0.1

[a]Control diet contained 3% corn oil. CLA1 and CLA2 represent 2.5% corn oil +0.5% CLA or 2% corn oil + 1% CLA. [a–c]Means ± SD with different superscripts within a row differ significantly ($P < 0.05$) ($n = 5$). For abbreviations, see Table 1. Total SFA also includes 16:0 dimethylacetal (DMA) and 18:0 DMA. Total MUFA includes 16:1, 18:1, 20:1, and 24:1.

through alteration in n-6 and n-3 FA ratio, may all contribute to embryonic health and survival in CLA-enriched eggs.

Enrichment of yolk with CLA did not affect the carcass weight, carcass dry matter, or ash content (Table 5). However, chick carcasses from CLA1 and CLA2 were lower in total fat than in Control ($P < 0.05$). The carcass fat for CLA2 chicks was 26% less than Control chicks. Latour *et al*. (16) reported an increase in the retention of remnant yolk in chicks hatched from eggs containing CLA. Inhibition of yolk fat absorption or transfer through the yolk sac or an increase in oxidation may contribute to the reduced fat accumulation in CLA1 and CLA2 chicks. The beneficial effect of CLA in reducing body fat mass has attracted great attention and has been reported in various species (2,3). Feeding CLA at 2 or 3% has been associated with reduced carcass fat in broilers (27). The moisture and ash contents of chick carcasses in our study were not affected by maternal dietary CLA, in agreement with studies reported in broiler chickens fed diets containing 2 or 3% CLA (27).

TABLE 5
Carcass Characteristics of Newly Hatched Chicks Hatched from Breeder Hens Fed Diets with or without CLA[a]

	Dietary treatments		
	Control	CLA1	CLA2
Carcass weight (g)	36.0 ± 0.6	35.1 ± 0.9	34.1 ± 0.8
Carcass total fat (%)	6.1 ± 0.9[a]	5.1 ± 0.8[b]	4.5 ± 0.3[b]
Carcass dry matter (%)	25.7 ± 1.6	28.1 ± 3.9	27.1 ± 0.7
Carcass ash (%)	2.5 ± 0.2	2.6 ± 0.3	2.5 ± 0.3

[a]Control diet contained 3% corn oil. CLA1 and CLA2 represent 2.5% corn oil + 0.5% CLA or 2% corn oil + 1% CLA. [a–b]Means ± SD with different superscripts within a row differ significantly ($P < 0.05$) ($n = 5$).

Poor hatchability and an increase in culls are major economic losses to hatching egg producers. Owing to the current interest in increasing the CLA content of ruminant and nonruminant foods, fats and FA from rendered sources may enter the livestock and avian feed chain. Inclusion of such rendered fat in the diets of breeder birds and egg-laying species may affect the health of the progeny. The results from the present study demonstrating that increasing yolk CLA alters lipid metabolism in chicks suggest that further investigations are needed on the use of CLA in breeder animal feeding and also on the long-term clinical use of CLA supplementation in lactating women.

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