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Effect of an Oil Byproduct from Conjugated Linoleic Acid (CLA) Purification on CLA Accumulation and Lipogenic Gene Expression in Broilers

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A previous study showed that supplementing broilers with an oil byproduct obtained during the purification process of conjugated linoleic acid (CLA) from safflower oil could result in CLA-enriched egg yolks more efficiently than feeding purified CLA (free fatty acid form). On this basis, this study evaluated whether dietary CLA byproduct (CBP) supplementation would enhance CLA accumulation in broiler muscle and its lipogenic mRNA expression in the liver. A total of 456 1-day-old male broiler chicks were randomly assigned to four groups, each of which was given one of the following 2% dietary supplements for 4 weeks: soybean oil (control), safflower oil (SAF), purified CLA, and CBP. During the feeding trial, little alteration in broiler performance was observed among the test groups. CLA accumulation efficiency in the breast muscle did not differ significantly between the CLA- and CBP-fed groups after feeding of the test diet for 3 weeks. CLA supplementation also induced lipogenesis in the livers of the broilers, and it significantly increased the relative mRNA levels of sterol regulatory element binding protein 1 (SREBP1), as well as its target genes: fatty acid synthase (FAS) and acetyl coenzyme A carboxylase (ACC) ($p < 0.05$). However, in the CBP-fed group, SREBP1 and ACC mRNA levels were not significantly different from the controls ($p > 0.05$). These results suggest that CBP could be an efficient dietary source that promotes CLA accumulation in broiler muscle without inducing lipogenesis in the liver or compromising performance and meat quality in the birds.

KEYWORDS: Conjugated linoleic acid; byproduct; meat quality; lipogenesis; broiler

INTRODUCTION

Conjugated linoleic acid (CLA) refers to a group of positional and geometric linoleic acid isomers with various conjugated double-bond arrangements. CLA plays a variety of preventive roles against degenerative diseases such as cancer (*1, 2*) and atherosclerosis (*3*). CLA also reduces the catabolic effect associated with immune stimulation (*4*), and it improves the protein to fat ratio (*5*) in several animal models. In addition, many studies of CLA-associated metabolic effects in various animals and adipocyte cultures suggest that CLA directly affects lipogenesis at the levels of gene expression and major enzyme activities (*6*−*8*). For this reason, considerable efforts have focused on producing CLA-enriched animal products by manipulating feeding regimens (*9, 10*). However, applying CLA as a feed additive is limited due to its high purification cost, which necessitates acquiring an economic alternative in order to elevate CLA in food products to a physiologically effective level.

Generally, the purification process for the production of high-purity CLA is performed using urea-inclusion crystallization with crude CLA (*11*). During this process, the crude CLA, which is not contained in crystallized urea, is converted to esterified forms, yielding a CLA byproduct (CBP) that still contains high

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levels of CLA (up to 80%). To eliminate this conspicuous waste, we proposed the utilization of this byproduct as a functional feed additive, although there has been no precedent for such an application. In our previous study, dietary supplementation with CBP more efficiently promoted CLA accumulation in egg yolks than did supplementation with purified CLA, and CBP did not cause adverse effects on egg quality. We attributed this distinct difference to high levels of the esterified form of CLA in the CBP oil (*12*).

In support of this idea, the form of digested fats may influence the composition of the chylomicrons they eventually form, because triglycerides (TG) are resynthesized in the intestinal mucosa using monoglycerides from dietary lipids (*13*). Because the esterified forms of fatty acids (especially those in the *sn*-2 position of monoglycerides) favor the synthesis of chylomicrons in the intestinal cells, CLA absorption into the epithelial cells of animals would be more active after consumption of the esterified form than after consumption of the free form. However, differences among species or target tissues should be considered when one is drawing parallels from such an observation.

There have been numerous reports on how dietary CLA affects fatty acid metabolism in mammals, but only a few have focused on birds (*7, 14*). Moreover, efforts to overcome the limits of dietary CLA consumption using novel CLA alternatives have been rare. Therefore, the objective of this study was to determine the effects of dietary CBP supplementation on meat quality and fatty acid composition in broiler chicks by measuring changes in lipogenesis within their livers. We monitored their relative mRNA levels of sterol regulatory element binding protein 1 (SREBP1), fatty acid synthase (FAS), acetyl coenzyme A carboxylase (ACC), and malic enzyme in the liver, as well as their blood immune responses. Our results revealed a potentially valuable outlet for oil byproduct from fatty acid purification procedures, and they demonstrated that such a byproduct could be utilized as a cost-effective source for enriching animal tissues with functional molecules.

MATERIALS AND METHODS

Birds and Experimental Design. A total of 456 1-day-old male broiler chicks (Ross × Ross) were randomly distributed into four groups, each of which received a 2% dietary supplementation of one of the following: soybean oil (control), safflower oil (SAF), purified CLA (CLA), or CBP (CBP) (Lipozen Inc., Pyongtaek, Korea). The ingredients and chemical compositions of the experimental diets are shown in **Table 1**. **Table 2** presents the fatty acid composition of the oils used in the feeding trial. To determine the optimum feeding period for the test samples, all birds within each test group were reassigned to three groups in which they were fed the test diet during different periods, respectively, while the birds in the control group were fed only the control diet for 4 weeks. The experimental design is schematically represented in **Figure 1**. The birds were allowed ad libitum access to feed and water and were maintained on a 24-h constant-light program. Body weights and feed consumption were recorded every week for the entire study. After 28 days of feeding, blood samples were obtained from the brachial veins, and five birds per group were sacrificed by neck cutting. Liver samples were excised and weighed, followed by immediate snap freezing in liquid N_2. Aliquots of the liver samples designated for RNA isolation were stored at −70 °C. The birds were scalded at 50−52 °C for approximately 30 s and then placed in a rotary drum plucker for 30 s to remove the feathers. The carcasses, including the breast muscle, legs, liver, and abdominal fat, were then excised and weighed individually, after which the birds were processed by removing the head, neck, and shanks. Meat quality measurements were performed with the breast muscle samples, and aliquots of the breast meat designated for lipid analysis were stored at −20 °C until use. All animal-based procedures were in accordance with Korea University's "Guidelines for the Care and Use of Experimental Animals."

Table 1. Composition of the Basal Diets for Broilers

item	starter diet, days 1−14	grower diet, days 15−28
ingredients, %		
corn grain	57.84	63.49
corn gluten meal	9.30	5.20
soybean meal	21.60	21.60
fish meal (60%)	4.18	2.70
wheat bran	0.40	0.40
tallow	3.67	3.73
limestone	1.60	1.60
tricalcium phosphate	0.50	0.45
L-lysine	0.18	0.10
salt	0.25	0.25
vitamin−mineral mixture[a]	0.25	0.25
choline	0.22	0.22
chemical composition, %[b]		
crude protein	23.09	20.09
lysine	1.21	1.05
methionine	0.50	0.41
Ca	1.02	0.93
non-phytate P	0.57	0.51
ME, kcal/kg	3200	3200

[a] Mixture contains following amounts of micronutrients per kg: vitamin A, 1,600,000 IU; vitamin D3, 300,000 IU; vitamin K3, 130 mg; vitamin B2, 1000 mg; niacin, 2000 mg; calcium pantothenate, 800 mg; folic acid, 60 mg; DL-methionine, 6000 mg; Mn, 12000 mg; Zn, 9000 mg; Fe, 4000 mg; Cu, 500 mg; I, 250 mg; Co, 100 mg; Ca, 7140 mg; BHT, 6000 mg. [b] Calculated values.

Table 2. Fatty Acid Compositions of Oils Used in Feeding Trial (Percent of Fat)

	dietary group[a]			
fatty acid	CON	SAF	CLA	CBP
C16:0	10.90	5.77	4.10	4.24
C16:1	0.08	nd[b]	nd	nd
C18:0	3.84	2.26	1.49	2.20
C18:1	23.9	11.33	10.68	9.90
C18:2	52.3	78.7	0.98	0.65
C18:3	5.74	nd	nd	nd
CLA *c*-9,*t*-11	nd	nd	38.00	34.57
CLA *t*-10,*c*-12	nd	nd	38.16	35.65
CLA *c*-9,*c*-11	nd	nd	1.16	2.17
CLA *c*-10,*c*-12	nd	nd	1.23	1.68
CLA *t*-9,*t*-11	nd	nd	0.30	0.85
CLA *t*-10,*t*-12	nd	nd	0.37	1.10
total CLA	nd	nd	79.22	76.02

[a] CON, soybean oil 2%; SAF, safflower oil 2%; CLA, purified CLA 2%; CBP, CLA byproduct 2%. [b] nd, not detected.

Meat Quality Measurements. *Meat Color and pH Measurements.* The color of each breast meat sample was determined using a chromameter (model CR-210, Minolta Co.) and reported using values from the CIE system: lightness (L^*), redness (a^*), and yellowness (b^*). The color of each breast was measured in duplicate, with one reading in the anterior portion and one reading in the posterior portion of the muscle. All color readings were taken on the skin side surface in an area free of obvious color defects resulting from overscalding, bruises, or blood accumulation. The pH of the breast meat was measured at 20 min post-mortem with a pH-meter equipped with a pH-electrode (NWKbiner pH, K-21, Landsberg, Germany). The electrode was inserted for 10 s, approximately 2.5 cm below the surface of the anterior portion of the meat.

Drip Loss Measurements. A 4-g sample, 1.5 cm in diameter and approximately 4 cm in length, was cored from the breast meat sample perpendicular to the length of the meat and then suspended in a plastic bag for 7 days. The sample was weighed at 1, 3, 5, and 7 days.

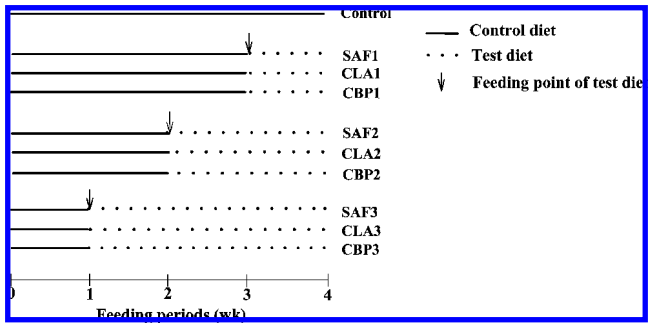

Figure 1. Experimental design for determining the optimum test diet feeding period. Control, soybean oil (2%); SAF, safflower oil (2%); CLA, purified CLA oil (2%); CBP, CLA byproduct oil (2%). Birds in each test group were assigned to three groups and fed the test diet for the indicated periods, whereas birds in the control group were fed the control diet for 4 weeks.

TBARS Measurements. A 5-g muscle sample was weighed, placed into a 50-mL test tube, and homogenized with 15 mL of deionized distilled water using a Polytron homogenizer 6 (type PT 10/35) for 10 s at the highest speed. Subsequently, 1 mL of muscle homogenate was transferred to a disposable test tube (3 × 100 mm). Butylated hydroxyanisole (50 μL, 7.2%) and thiobarbituric acid/trichloroacetic acid (2 mL) were added, and the mixture was vortexed and incubated in a boiling-water bath for 15 min to develop the color. The sample was then cooled in cold water for 10 min, vortexed again, and centrifuged for 15 min at 2000*g*. The absorbance of the resulting supernatant solution was determined at 531 nm against a blank containing 1 mL of deionized distilled water and 2 mL of thiobarbituric acid/trichloroacetic acid solution. The amounts of thiobarbituric acid-reactive substances (TBARS) were expressed as milligrams of malondialdehyde per kilogram of meat.

Water-Holding Capacity Measurements. Water-holding capacity was estimated by determining the amount of expressible juice in the meat, using a modification of the following filter paper press method described by Wierbicki and Deatherage (*15*): A meat sample weighing between 200 and 400 mg was placed on an 11-cm diameter filter paper between plexiglass plates and pressed at 2000 psi for 1 min. The outline area of the expressible juice and the meat film was traced, and the two areas were determined using a compensating polar planimeter. The expressible juice was expressed as a percentage, calculated as follows:

$$\text{expressible juice \%} = [100 \times (\text{total juice area} - \text{muscle film area}) \times \text{water/square inch filter}] / [\text{total moisture (mg) of original sample (sample wt in mg)} \times \text{\% moisture}] \quad (1)$$

A higher expressible juice percentage correlates with decreased water-holding capacity.

Blood Characteristics. White blood cell, red blood cell, and lymphocyte counts from whole blood samples were determined with an automatic blood analyzer (ADVIA 120, Bayer). The blood samples were centrifuged at 2000*g* (4 °C) for 30 min to separate the serum. Serum total protein and albumin were determined with an automatic biochemistry analyzer (Hitachi 747).

Fatty Acid Analyses. All chemicals used for GC analysis were of analytical grade and purchased from Sigma (St. Louis, MO). Lipids from the breast meat aliquots were extracted with hexane/isopropanol (3:2, v/v). The fatty acids were then converted to methyl esters, as described in our previous study (*12*). Briefly, 0.5 mL of toluene and 2 mL of 5% KOH−MeOH were added to the lipids, and the samples were vortex-mixed, heated at 70 °C for 8 min, and cooled in cold water. The samples then received 2 mL of 14% BF_3−MeOH and were heated at 70 °C for an additional 8 min. The samples were cooled, 3 mL of 5% NaCl was added to the sample and mixed, and 5 mL of distilled water and 0.5 mL of hexane were added to extract the fatty acid methyl esters. The mixtures were vortexed and centrifuged at 5000*g* for 10 min, and the upper phase was collected and dried with sodium sulfate. The samples were analyzed for total fatty acids, including CLA isomers, using an HP7890 gas chromatograph with a flame ionization detector (Hewlett-Packard). The fatty acid methyl esters were separated using a Supelcowax-10 fused silica capillary column (100 m × 0.32 mm i.d., 0.25 μm film thickness; Supelco, Inc., Bellefonte, PA) with a 1.2 mL/min helium flow rate. The GC was operated at a temperature of 190 °C for 5 min, followed by heating at 3 °C/min to 240 °C and holding for 30 min. Both the injector and detector were maintained at 260 °C. The sample (1 μL) was injected into the column in the split mode (50:1). The peak of each CLA isomer (*cis*-9,*trans*-11; *trans*-10,*cis*-12; *cis*-9,*cis*-11; *cis*-10,*cis*-12; *trans*-9,*trans*-11; *trans*-10,*trans*-12) and other fatty acids were identified and quantified by comparing their retention times and peak areas to those of each respective fatty acid standard (Sigma). Fatty acid content was expressed as the percent of total fatty acids. Heptadecanoic acid (C17:0) was included as an internal reference before lipid extraction to determine the recovery of the fatty acids in each sample.

RNA Extraction and Determination by Real-time PCR. The liver tissues were homogenized in 1 mL of TRIzol reagent, and RNA was isolated as described in the TRIzol protocol. The RNA was quantified spectrophotometrically using a spectrophotometer (NanoDrop). From each sample, 1 μg of total RNA was reverse transcribed to cDNA with a random primer using an RT-PCR high kit (Toyobo) in a total volume of 20 μL, as described in the RT-PCR high kit protocol. The cDNA samples obtained by the above-mentioned reverse transcription reaction were used in real-time PCR. The primer sets for each target gene [SREBP1 (GenBank accession no. NM204126), FAS (GenBank accession no. NM205155), ACC (GenBank accession no. NM205505), and malic enzyme (GenBank accession no. AF408407)] were designed with the Primer3 Out program. The amplification was performed in a total volume of 20 μL, including 3 μL of cDNA (150 ng), 1 μL of each primer (10 pmol/μL), 10 μL of Power SYBR Green Master Mix (AppliedBiosystems), and 5 μL of distilled water. The reactions proceeded in an ABI 7500 system as follows: 50 °C for 2 min, 94 °C for 2 min, and 45 cycles of 94 °C for 30 s, 62 °C for 30 s, and 72 °C for 1 min. All samples were also examined in parallel for β-actin, and the relative quantities of each gene were presented in terms of $2^{-\Delta\Delta Ct}$, calculated using the ΔCt (Ct value of target gene − Ct value of β-actin) and ΔΔCt values (ΔCt value of tested sample − ΔCt value of control sample).

Statistical Analyses. Statistical differences were determined by ANOVA, with mean separations performed by Duncan's multiple-range test using the general linear model procedure in the SAS statistical software package (SAS Institute Inc., Cary, NC). Samples were analyzed in quintuples, and the variation between samples was expressed as the pooled standard error of the mean (SEM) or mean ± SEM, when applicable. Contrast analysis was applied to compare the effects of diet and feeding period of test diet, and interaction between diet and feeding period was also examined.

RESULTS AND DISCUSSION

Growth Performance and Carcass Traits. During the feeding trial, none of the tested groups exhibited significant alterations in growth performance, although average daily gain (ADG) and average daily feed intake (ADFI) were generally higher in the experimental groups than in the control group (**Table 3**; $p > 0.05$). Badinga et al. (*14*) reported that weight gain decreased with the concentration of CLA in the diet. In contrast with this paper, Bolukbasi (*16*) demonstrated that broilers given 1, 2, or 3% CLA exhibited increased body weights and rates of body weight gain. There was no significant difference in ADG, ADFI, or ADFI/ADG (F/G) between the CLA and CBP groups, but safflower oil supplementation tended to decrease the ADFI as the feeding period progressed, and we observed a similar pattern with CLA and CBP supplementation. These results suggest that feeding broilers dietary lipid sources, such as CLA or CBP, did not greatly affect their growth performance during the period of this experiment, but they could act adversely when continued for a longer time.

Table 3. Effects of Different Oil Supplementations on Growth Performance in Broilers during Feeding Periods

	dietary group[a]														
	CON	SAF			CLA			CBP					effects[b]		
	0 weeks	1 week	2 weeks	3 weeks	1 week	2 weeks	3 weeks	1 week	2 weeks	3 weeks	SEM	*P* value	diet (D)	period (P)	D × P
ADG, g	35.31bc	37.94a	37.28a	36.87ab	36.64ab	36.86ab	36.77ab	38.04a	34.08c	37.52a	0.51	0.0094	ns	ns	ns
ADFI, g	56.97b	63.45a	61.82a	58.09ab	62.85ab	59.32ab	60.46ab	62.76ab	57.28b	60.31ab	1.66	0.0415	ns	*	ns
F/G	1.61	1.67	1.66	1.58	1.72	1.61	1.65	1.65	1.68	1.61	0.06	0.8308	ns	ns	ns

[a] Means with different letters within the same row are significantly different ($n = 24$ control group and $n = 48$ treatment group). CON, soybean oil 2%; SAF, safflower oil 2%; CLA, purified CLA 2%; CBP, CLA byproduct 2%. [b] Diet, CON vs SAF vs CLA vs CBP; Period, feeding period of each test diet; D × P, interaction among the diets. *, $p < 0.05$; ns, nonsignificant.

Table 4. Effects of Different Oil Supplementation on the Carcass Traits of Broilers (Percent)

	dietary group[a]														
	CON	SAF			CLA			CBP					effects[b]		
	0 weeks	1 week	2 weeks	3 weeks	1 week	2 weeks	3 weeks	1 week	2 weeks	3 weeks	SEM	*P* value	diet (D)	period (P)	D × P
breast	6.55	6.60	6.66	6.02	6.52	6.80	6.09	5.88	6.36	6.50	0.39	0.1308	ns	ns	ns
leg	2.84	3.39	3.06	2.96	3.00	3.18	3.22	3.28	2.97	3.11	0.22	0.9219	ns	ns	ns
liver	2.76b	3.28ab	3.10ab	3.02ab	3.48a	3.41ab	3.34ab	2.79b	3.05ab	3.11ab	0.20	0.0241	*	ns	ns
abdominal fat	1.43ab	1.46ab	1.30ab	1.85a	1.29ab	1.50ab	1.32ab	1.64ab	1.26b	1.55ab	0.17	0.0084	ns	ns	ns

[a] Means with different letters within the same row are significantly different ($n = 5$). CON, soybean oil 2%; SAF, safflower oil 2%; CLA, purified CLA 2%; CBP, CLA byproduct 2%. [b] Diet, CON vs SAF vs CLA vs CBP; period, feeding period of each test diet; D × P, interaction among the diets. *, $p < 0.05$; ns, nonsignificant.

Table 5. Effects of Different Oil Supplementation on the Breast Meat Qualities of Broilers

	dietary group[a]														
	CON	SAF			CLA			CBP					effects[b]		
	0 weeks	1 week	2 weeks	3 weeks	1 week	2 weeks	3 weeks	1 week	2 weeks	3 weeks	SEM	*P* value	diet (D)	period (P)	D × P
							Muscle Color[c]								
*L**	49.73b	42.92e	45.93de	45.70de	52.05ab	52.92a	46.45cd	49.29bc	48.92cd	46.36cd	1.03	0.0110	***	ns	***
*a**	14.09	16.92	15.37	15.50	14.95	16.34	16.17	16.66	16.19	15.06	0.94	0.4189	ns	ns	ns
*b**	13.10ab	11.07ab	10.68ab	10.29b	14.42a	12.43ab	9.48b	10.65ab	11.12ab	9.05b	1.24	0.0174	***	*	ns
							Drip Loss, Percent								
1 day	3.13	3.26	3.32	3.36	3.58	3.29	3.64	3.48	3.35	3.28	0.95	0.4189	ns	ns	ns
3 days	5.48	5.53	5.52	5.56	5.61	5.53	5.65	5.35	5.53	5.50	0.15	0.1336	ns	ns	ns
5 days	6.74	6.76	6.82	6.83	6.79	6.78	6.95	6.85	6.83	6.78	0.12	0.1547	ns	ns	ns
7 days	8.90	8.78	8.93	8.55	8.95	8.91	8.99	8.88	8.92	8.53	0.23	0.4916	ns	ns	ns
pH	6.16ab	6.28a	6.10bc	6.29a	6.04bc	6.11abc	6.15ab	5.96c	6.15ab	6.17ab	0.06	0.0107	*	ns	*
water-holding capacity, %	44.67abc	41.59bc	51.42ab	54.85a	46.12abc	43.46abc	43.53abc	41.54bc	37.90c	48.90abc	3.56	0.0238	ns	ns	ns
TBARS, MDA mg/kg of sample[d]	0.24a	0.20ab	0.21ab	0.12d	0.18bc	0.20ab	0.19abc	0.21ab	0.16bcd	0.15cd	0.02	0.0160	***	*	ns

[a] Means with different letters within the same row are significantly different ($n = 5$). CON, soybean oil 2%; SAF, safflower oil 2%; CLA, purified CLA 2%; CBP, CLA byproduct 2%. [b] Diet, CON vs SAF vs CLA vs CBP; period, feeding period of each test diet; D × P, interaction among the diets. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, nonsignificant. [c] L^* = lightness (greater value indicates a lighter color); a^* = redness (greater value indicates a redder color); b^* = yellowness (greater value indicates a more yellow color). [d] MDA, malondialdehyde.

The carcass yields of the breast, leg, liver, and abdominal fat in each group are shown in **Table 4**. There were no significant differences in the yields of the breast, leg, liver, and abdominal fat among the dietary groups ($p > 0.05$). In previous papers, 3% CLA supplementation significantly increased breast weight (*16*) and decreased liver weight (*17*) in broilers. However, we could not replicate this alteration with 2% CLA or CBP supplementation in the present study.

Meat Quality and Blood Characteristics. We observed changes in breast meat qualities, including color, drip loss, pH, water-holding capacity, and TBARS, after the 4-week feeding trial. Dietary CLA or CBP did not affect breast meat color compared with the control group when the broilers were fed the test diets for 1 week (**Table 5**), but L^* and b^* values decreased as the feeding period of test diet in both the CLA and CBP groups. In addition, the L^* and b^* values in the CBP group were lower than those in the CLA group when the broilers were fed the test diets for 1 week, but these differences became insignificant after 3 weeks of feeding ($p > 0.05$). In a previous study, the feeding of 2 or 3% commercial CLA (*c*-9,*t*-11:*t*-10,*c*-12 = 1:1) for 5 weeks decreased the L^*, a^*, and b^* values of cooked breast fillets, because feeding the birds CLA made the breast meat darker (*18*). Herein, however, the meat color did not reflect the CLA content in the muscle, because the CLA levels of the breast meat were lower when the broilers were fed CLA for 3 weeks than when they were fed CLA for 2 weeks (**Figure 2a**). Indeed, safflower oil supplementation caused the most significant decrease in the L^* value without resulting in CLA accumulation in the breast meat.

There were no significant differences in drip loss (at 1, 3, 5, or 7 days) or pH measurements of breast meat from broilers fed the different oils. These results correlate well with previous findings that up to 3% dietary CLA did not affect the pH of breast meat in broilers (*18*). In contrast, 3 weeks of safflower oil supplementation significantly increased the water-holding capacity. The TBARS values of meat samples from the CBP

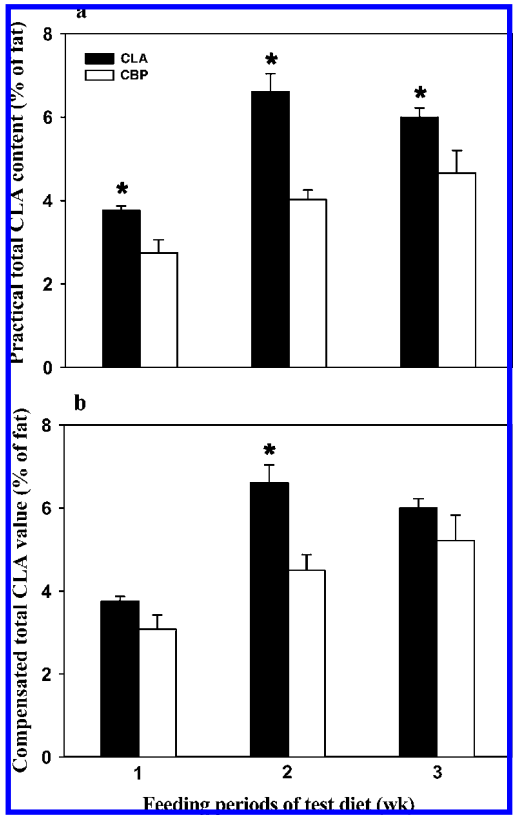

Figure 2. CLA accumulation in breast muscle by feeding different CLA sources: (**a**) practical total CLA levels; (**b**) compensated total CLA values based on the CLA levels in each test oil [CLA, purified CLA oil (2%); CBP, CLA byproduct oil (2%)]. Values with an asterisk are significantly different from the control values ($p < 0.05$). Error bars represent standard deviations from each data point ($n = 5$). Purified CLA oil = CLA 0.76 g/g of oil; CLA byproduct oil = CLA 0.67 g/g of oil; compensated total CLA value = practical total CLA level × 0.76/0.67. Total CLA values in (**b**) were compensated for content of total CLA in CBP fed to the broilers.

group were lower than those from the control group when the broilers were fed the test diets for 2 or 3 weeks. These data indicate that dietary CBP could reduce TBARS values in broiler muscle.

Supplementation of the various dietary oils did not affect levels of red blood cells, white blood cells, or albumin in the blood (**Table 6**), but induced increases in lymphocyte and serum total protein levels. Similarly, Lai et al. (*19*) observed a linear improvement of lymphocyte counts in serum, which increased with the dietary CLA level, and the authors recommended supplementation with 2% CLA to optimize the immune function of weaned pigs. Likewise, the results herein suggest that dietary CBP did not adversely affect biochemical blood characteristics, including red blood cell, white blood cell, lymphocyte, serum total protein, and albumin levels. Moreover, in light of these findings, the effects of CBP on broiler immune function should be examined more closely in future studies.

Table 7. Fatty Acid Compositions of Breast Meat after Feeding the Test Diets for 3 Weeks (Percent of Fat)

	dietary group[a]					
fatty acid[b]	CON	SAF	CLA	CBP	SEM	*P* value
C14:0	0.35	0.34b	0.55a	0.67a	0.01	0.0033
C15:0	2.65	3.72	3.42	2.87	0.89	0.4615
C16:0	18.04b	17.78b	21.45a	22.83a	1.48	0.0004
C16:1	3.11	2.40	3.00	2.88	0.37	0.4899
C18:0	10.40c	12.02b	14.58a	11.81b	0.60	0.0003
C18:1 *t*-11	0.37b	0.42b	0.59a	0.61a	0.00	0.0002
C18:1 *c*-9	20.80a	15.90b	13.04c	18.38ab	2.27	0.0003
C18:2	21.05a	21.74a	20.59ab	18.86b	1.26	0.0324
C18:3	0.86a	0.35c	0.45bc	0.58b	0.01	0.0003
CLA *c*-9,*t*-11	nd[c]	nd	3.10a	1.84b	0.06	<0.0001
CLA *t*-10,*c*-12	nd	nd	2.91	2.81	0.07	<0.0001
C20:1	0.40a	0.35ab	0.26c	0.32bc	0.00	0.0089
C20:2	0.77b	1.26a	0.63c	0.44d	0.01	<0.0001
C20:3	0.11a	0.10ab	0.05b	0.06ab	0.00	0.0478
C20:4	6.62b	8.55a	3.66c	3.46c	1.19	0.0003
C20:5	0.58bc	0.43c	0.93a	0.73ab	0.02	0.0078
SFA	31.44c	33.86b	40.00a	38.17a	2.03	<0.0001
MUFA	24.68a	19.06bc	16.90c	22.18ab	3.40	0.0012
PUFA	29.99b	32.42a	32.32a	28.80b	1.77	0.0114
n-6 PUFA	28.55b	31.64a	30.94a	27.48b	1.67	0.0053
n-3 PUFA	1.44a	0.77b	1.38a	1.31a	0.01	<0.0001
C18:0/C18:1	0.49c	0.74b	1.07a	0.62bc	0.01	0.0001
total CLA	nd	nd	6.00a	4.66b	0.27	<0.0001

[a] Means with different letters within the same row are significantly different ($n = 5$). CON, soybean oil 2%; SAF, safflower oil 2%; CLA, purified CLA 2%; CBP, CLA byproduct 2%. [b] SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid. [c] nd, not detected.

Fatty Acid Profiles of Breast Meat. The breast meat fatty acid compositions were analyzed after the 4-week feeding trial. Overall, total CLA levels in the breast meat were higher in the test group fed the purified CLA oil than in meat from the CBP group (**Figure 2a**). In the CLA group, the total CLA levels in the breast meat peaked after feeding of the test diet for 2 weeks, whereas CBP supplementation resulted in the highest CLA accumulation (5% of total fat) after 3 weeks of feeding. In addition, the CLA and CBP oils did not show significant differences in the efficiency by which they promoted CLA accumulation in the breast meat when the broilers were fed for 3 weeks (**Figure 2b**). These results suggest that utilizing CBP as a source for promoting CLA accumulation in broilers could have a sufficient economic advantage over purified CLA oil.

These findings, however, were not fully consistent with our previous results for laying hens (*12*). CBP supplementation

Table 6. Effects of Different Oil Supplementation on the Blood Characteristics of Broilers

	dietary group[a]														
	CON	SAF			CLA			CBP					effects[b]		
	0 weeks	1 week	2 weeks	3 weeks	1 week	2 weeks	3 weeks	1 week	2 weeks	3 weeks	SEM	*P* value	diet (D)	period (P)	D × P
red blood cells, $\times 10^6/\mu$L	2.37ab	2.50ab	2.49ab	2.23abc	1.94c	2.55a	2.46ab	2.38ab	2.24abc	2.12bc	0.12	0.0348	ns	ns	ns
white blood cells, $\times 10^3/\mu$L	367.80abc	426.58bc	428.70ab	384.70a	329.98abbc	420.98abc	403.38abc	393.75bc	371.60c	365.43abc	0.04	0.0423	ns	ns	ns
lymphocytes, %	58.25ab	69.00ab	66.75ab	71.75a	67.50ab	65.50ab	57.25b	69.75ab	67.67ab	69.00ab	4.12	0.0035	ns	ns	ns
total protein, g/dL	2.88c	3.28abc	3.30abc	3.13bc	3.08bc	3.35ab	3.18abc	3.18abc	3.60a	3.30abc	0.13	0.0421	**	ns	ns
albumin, g/dL	1.33	1.45	1.53	1.48	1.35	1.48	1.40	1.43	1.53	1.48	0.06	0.4757	*	ns	ns

[a] Means with different letters within the same row are significantly different ($n = 5$). CON, soybean oil 2%; SAF, safflower oil 2%; CLA, purified CLA 2%; CBP, CLA byproduct 2%. [b] Diet, CON vs SAF vs CLA vs CBP; period, feeding period of each test diet; D × P, interaction among the diets. *, $p < 0.05$; **, $p < 0.01$; ns, nonsignificant.

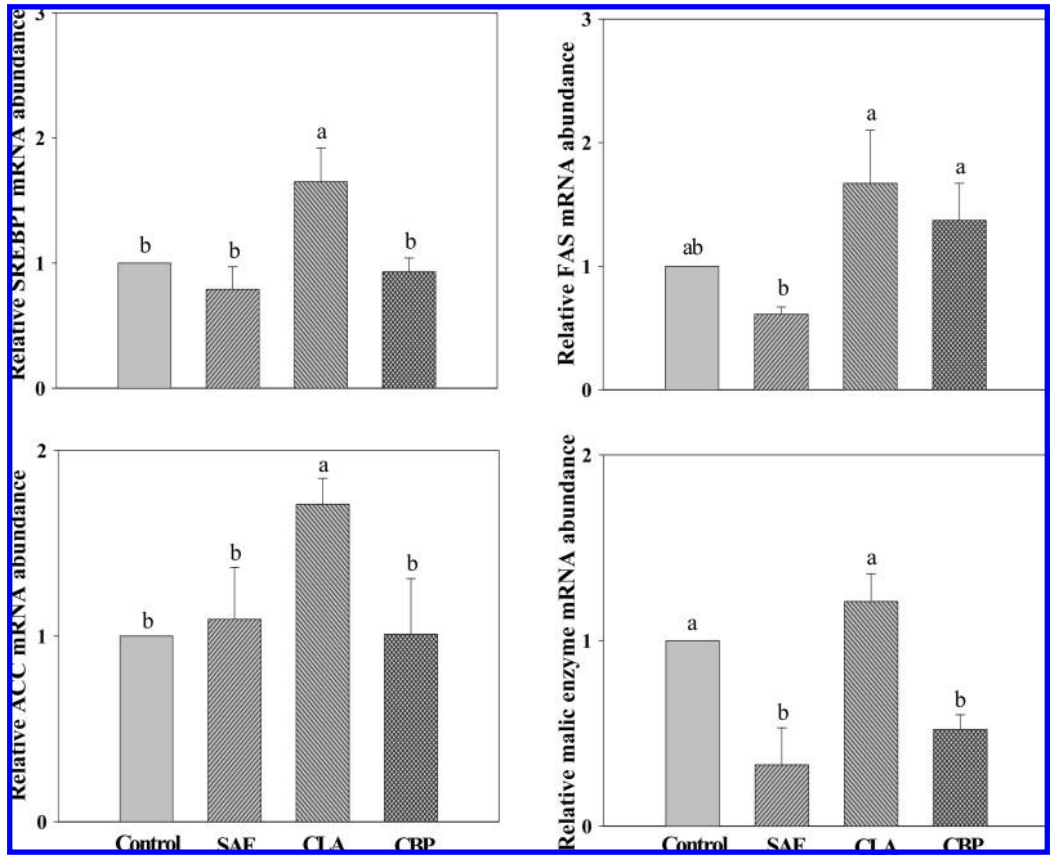

Figure 3. Changes in mRNA abundance of hepatic lipogenic genes in broilers after feeding the test diets for 3 weeks. Control, soybean oil (2%); SAF, safflower oil (2%); CLA, purified CLA oil (2%); CBP, CLA byproduct oil (2%). Bars with different letters are significantly different from controls ($p < 0.05$). Error bars represent standard deviations from each data point ($n = 5$). Relative quantities of each gene are represented as the $2^{-\Delta\Delta Ct}$, calculated using the ΔCt (Ct value of target gene − Ct value of β-actin) and ΔΔCt values (ΔCt value of tested sample − ΔCt value of control sample). The value of the control group was set at 1.

increased the total CLA levels more efficiently in egg yolks than purified CLA, and we demonstrated that the CLA within CBP, which was largely in esterified forms, could more easily incorporate into egg yolks than the free form of CLA, due to its superior absorption rate in bird intestines. Although we cannot yet fully explain this difference between the broiler breast meat and egg yolks, it is likely that the difference is closely related to variations in lipid utilization during chick development. In fact, yolk-derived lipids during chick development are important, because the embryo derives >90% of its energy requirements from the oxidation of the yolk lipids (*20*). Additionally, Latour et al. (*21*) reported that yolk fatty acids are utilized and regulated in a specific manner during embryogenesis.

When the broilers were fed with CLA, the *c*-9,*t*-11 CLA isomers accumulated in the breast meat in slightly higher amounts than did the *t*-10,*c*-12 isomers (**Table 7**). The *c*-9,*t*-11 CLA isomer, which exists as a major form in nature, is preferentially incorporated into the membrane phospholipids fraction, which is a possible locus of action along the related signal transduction pathways. Other supplementation studies using broilers or hens have reported similar results (*16*, *22*), but our results clearly showed that the *t*-10,*c*-12 isomers preferentially incorporated into the breast meat when the broilers were treated with CBP, despite similar levels of the two CLA isomers in CBP. On the basis of these results, we propose that the fatty acid form could influence the differential deposition of CLA isomers into muscle tissues. We speculate the *t*-10,*c*-12 isomer predominates at the *sn*-2 position, because the *sn*-2 position fatty acids in TG are more easily absorbed into the intestine, whereas no preference exists between the fatty acids in the *sn*-1 and *sn*-3 positions (*13*). It is necessary to confirm through further studies whether the *t*-10,*c*-12 isomer in CBP preferentially incorporates at the *sn*-2 position in broiler muscle tissue.

In rodents, CLA decreases the activity of hepatic stearoyl-coenzyme A desaturase (SCD), which catalyzes the insertion of a double bond between the C9 and C10 atoms of C16:0 and C18:0 aliphatic chains, in order to form C16:1 and C18:1 chains, respectively. In general, *t*-10,*c*-12 CLA is known to be actively involved in the gene expression of fat-metabolizing enzymes. Lin et al. (*23*) reported that *t*-10,*c*-12 CLA significantly decreased the activity and mRNA abundance of SCD in mammary of lactating mice compared to *c*-9,*t*-11 CLA, but there was no difference in hepatic activity and mRNA abundance of SCD. We observed that 3 weeks of CLA supplementation significantly decreased monounsaturated fatty acid levels in the breast meat, mainly due to decreased C18:1 *c*-9 levels. Similar changes were also observed in the SAF group. These results correlated to previous findings (*24*, *25*) that demonstrated polyunsaturated fatty acids (PUFA) can down-regulate mRNA levels of lipogenic genes such as SCD. On the other hand, the C18:1 *c*-9 levels in the CBP group were not significantly different from those in the control group, which mirrored our results found in egg yolks obtained from laying hens that had been fed a CBP-supplemented diet (data not shown). Although we could not obtain supportive data on SCD gene expression in the present study, the fatty acid analysis results suggest that dietary CBP supplementation could promote CLA accumulation in broilers with little adverse effects on the lipid composition of their tissues.

Therefore, it is reasonable to conclude that CBP, which has previously been regarded as waste, can be efficiently utilized as a chicken feed supplement to promote the accumulation of CLA in broiler tissue without adversely affecting broiler performance and meat quality.

Expression of Lipogenic Genes in the Liver. SREBP1 is a transcription factor that stimulates the expression of mammalian lipogenic genes that contain a sterol regulatory element in their promoter regions. These genes, which are involved in hepatic triglyceride synthesis, include FAS, ACC, and malic enzyme. In mammals, dietary PUFA decrease the expression of these genes (*26*), but many researchers have reported various conflicting results regarding how CLA influences lipogenic metabolism in mammals. FAS and ACC activities dropped in sow mammary glands after the animals were fed CLA diets (*27*). Dietary CLA also reduced the expression of lipogenic genes in the adipose tissue of neonatal pigs (*28*). In contrast to these findings, Takahashi et al. (*29*) demonstrated that CLA did not reduce, but rather increased, SREBP1 transcription in mammalian liver cells. Likewise, CLA supplementation increased SREBP1 mRNA levels and hepatic lipogenic gene expression in the present study (**Figure 3**). Du and Ahn (*7*) indicated that dietary CLA reduced lipogenesis in adipose tissues and mammary glands, but not in the liver, and this property could be the reason that CLA is ineffective in reducing fat accumulation in birds, in which the liver is the primary site of lipogenesis. This increase of hepatic lipogenesis by CLA may relate to its action on the mitochondrial citrate transport protein activity. Hepatic lipogenesis implies a complex series of reactions occurring partly in the mitochondrial matrix and partly in the cytosol, and this functional connection between these two different cellular compartments is possible by the presence of a mitochondrial transport protein, the tricarboxylate or citrate carrier. Ferramosca et al. (*30*) reported that CLA administration to mice could induce hepatic lipogenesis by stimulating the synthesis and membrane insertion of citrate carrier in liver mitochondria.

However, CBP supplementation significantly suppressed SREBP1, ACC, and malic enzyme mRNA levels in the liver compared with CLA supplementation ($p < 0.05$). In addition, these gene expression levels in the CBP group were not significantly different compared to the control group (2% soybean oil), and the malic enzyme mRNA abundance was rather significantly decreased ($p < 0.05$). In a previous study, feeding rats with a soybean oil diet significantly decreased hepatic lipogenic enzyme activity (*31*). Although we did not definitively confirm that soybean oil reduced the mRNA levels of lipogenic genes, it is clear that they may have been influenced to some degree by soybean oil supplementation. The increased lipogenesis raised TG levels in the liver and could eventually induce fatty liver, a condition commonly associated with various metabolic syndromes such as diabetes and, in particular, with considerable growth restraint and mortality in broilers. Therefore, these results suggest that, in broilers, dietary CBP could prevent the formation of fatty liver, which could be induced by CLA supplementation, by reducing hepatic lipogenesis. Furthermore, it is notable that the mRNA expression levels of the lipogenic genes in the CBP group were similar to those in the SAF group, except for FAS. However, we observed no significant differences in liver weight among all tested groups after the 4-week feeding trial.

Porsgaard et al. (*32*) reported that the form of CLA did not differentially affect hamster plasma and liver TG concentrations, and Terpstra et al. (*33*) showed that CLA in the form of free fatty acid or TG had similar effects on body composition and energy balance in mice. However, the present results imply that the form of CLA may differently affect hepatic lipogenesis. The presence of minor constituents in CBP such as glycerol that are derived from saponification procedure of TG form of safflower oil would not significantly affect the results obtained with CBP, especially the gene expression of fat-metabolizing enzymes. Further studies using various animal models will be necessary to establish the physiological mechanism by which different sources of CLA regulate lipogenic metabolism.

LITERATURE CITED

(1) Belury, M. A.; Nickel, K. P.; Bird, C. E.; Wu, Y. M. Dietary conjugated linoleic acid modulation of phorbol ester skin tumor promotion. *Nutr. Cancer* **1996**, *26*, 149–157.

(2) Lee, K. W.; Lee, H. J.; Cho, H. Y.; Kim, Y. J. Role of the conjugated linoleic acid in the prevention of cancer. *Crit. Rev. Food Nutr*. **2005**, *45*, 135–144.

(3) McLeod, R. S.; LeBlanc, A. M.; Langille, M. A.; Mitchell, P. L.; Currie, D. L. Conjugated linoleic acids, atherosclerosis, and hepatic very-low-density lipoprotein metabolism. *Am. J. Clin. Nutr*. **2004**, *79*, 1169–1174.

(4) Kelley, D. S.; Warren, J. M.; Simon, V. A.; Bartolinin, G.; Mackey, B. E.; Erickson, E. L. Similar effects of c9,t11-CLA and t10-c12-CLA on immune cell functions in mice. *Lipids* **2002**, *37*, 725–728.

(5) Simona, O.; Mannera, K.; Schafera, K.; Sagredosb, A.; Ederc, K. Effects of conjugated linoleic acids on protein to fat proportions, fatty acids, and plasma lipids in broilers. *Eur. J. Lipid Sci. Technol*. **2000**, *102*, 402–410.

(6) Corino, C.; Mourot, J.; Magni, S.; Pastorelli, G.; Rosi, F. Influence of dietary conjugated linoleic acid on growth, meat quality, lipogenesis, plasma leptin and physiological variables of lipid metabolism in rabbits. *J. Anim. Sci*. **2002**, *80*, 1020–1028.

(7) Du, M.; Ahn, D. U. Dietary CLA affects lipid metabolism in broiler chicks. *Lipids* **2003**, *38*, 505–511.

(8) Konig, B.; Spielmann, J.; Haase, K.; Brandsch, C.; Kluge, H.; Stangl, G. I.; Eder, K. Effects of fish oil and conjugated linoleic acids on expression of target genes of PPARa and sterol regulatory element-binding proteins in the liver of laying hens. *Br. J. Nutr*. **2008**, *100*, 355–363.

(9) Watkins, B. A.; Feng, S.; Strom, A. K.; Devitt, A. A.; Yu, L.; Li, Y. Conjugated linoleic acids alter the fatty acid composition and physical properties of egg yolk and albumen. *J. Agric. Food Chem* **2003**, *51*, 6870–6876.

(10) Kim, J. H.; Hwangbo, J.; Choi, N. J.; Park, H. G.; Yoon, D. H.; Park, E. W.; Lee, S. H.; Park, B. K.; Kim, Y. J. Effect of dietary supplementation with conjugated linoleic acid, with oleic, linoleic, or linolenic acid, on egg quality characteristics and fat accumulation in the egg yolk. *Poult. Sci*. **2007**, *86*, 1180–1186.

(11) Kim, Y. J.; Lee, K. W.; Lee, S.; Kim, H.; Lee, H. J. The production of high-purity conjugated linoleic acid (CLA) using two-step urea-inclusion crystallization and hydrophilic arginine−CLA complex. *J. Food Sci*. **2003**, *68*, 1948–1951.

(12) Kim, J. H.; Choi, N. J.; Park, H. G.; Kim, I. H.; Lee, H. G.; Song, M. K.; Whang, K. Y.; Kim, Y. J. Utilization of oil by-product from the purification process of conjugated linoleic acid as feeding supplements for the accumulation of conjugated linoleic acid in the egg yolk. *Poult. Sci*. **2008**, *87*, 64–70.

(13) Ramirez, M.; Amate, L.; Gil, A. Absorption and distribution of dietary fatty acids from different sources. *Early Hum. Dev*. **2001**, *65*, 95–101.

(14) Badinga, L.; Selberg, K. T.; Dinges, A. C.; Comer, C. W.; Miles, R. D. Dietary conjugated linoleic acid alters hepatic lipid content and fatty acid composition in broiler chickens. *Poult. Sci*. **2003**, *82*, 111–116.

(15) Wierbicki, E.; Deatherage, F. E. Water content of meats, determination of water-holding capacity of fresh meats. *J. Agric. Food Chem*. **1958**, *6*, 387–392.

(16) Bolukbasi, S. C. Effect of dietary conjugated linoleic acid (CLA) on broiler performance, serum lipoprotein content, muscle fatty acid composition and meat quality during refrigerated storage. *Br. Poult. Sci*. **2006**, *47*, 470–476.
(17) Bolukbasi, S. C.; Erhan, M. K. Effect of semi replacement of dietary olive oil and corn oil with conjugated linoleic acid on broiler performance, serum lipoprotein levels, fatty acid composition in muscles and meat quality during refrigerated storage. *J. Anim. Vet. Adv*. **2007**, *6*, 262–266.
(18) Du, M.; Ahn, D. U. Effect of dietary conjugated linoleic acid on the growth rate of live birds and on the abdominal fat content and quality of broiler meat. *Poult. Sci*. **2002**, *81*, 428–433.
(19) Lai, C. H.; Yin, J. D.; Li, D.; Liu, M. X. Effects of dietary conjugated linoleic acid supplementation on performance and immune function of early weaned pigs. *Chin. J. Anim. Sci*. **2004**, *40*, 9–12.
(20) Romanoff, A. L. *The Avian Embryo*; New York: MacMillan Publishing, 1960.
(21) Latour, M. A.; Devitt, A. A.; Meunier, R. A.; Stewart, J. J.; Watkins, B. A. Effect of conjugated linoleic acid. 1. Fatty acid modification of yolks and neonatal fatty acid metabolism. *Poult. Sci*. **2000**, *79*, 817–821.
(22) Yang, L.; Huang, Y.; Wang, H. Q.; Chen, Z.-Y. Isomeric distribution of conjugated linoleic acids (CLA) in the tissues of layer hens fed a CLA diet. *J. Agric. Food Chem* **2003**, *51*, 5654–5660.
(23) Lin, X.; Loor, J. J.; Herbein, J. H. *Trans*10,*cis*12−18:2 is a more potent inhibitor of de novo fatty acid synthesis and desaturation than *cis*9,*trans*11−18:2 in the mammary gland of lactating mice. *J. Nutr*. **2004**, *134*, 1362–1368.
(24) Sessler, A. M.; Ntambi, J. M. Polyunsaturated fatty acid regulation of gene expression. *J. Nutr*. **1999**, *128*, 923–926.
(25) Lefevre, P.; Tripon, E.; Plumelet, C.; Douaire, M.; Diot, C. Effects of polyunsaturated fatty acids and clofibrate on chicken stearoyl-coA desaturase 1 gene expression. *Biochem. Biophys. Res. Commun*. **2001**, *280*, 25–31.
(26) Xu, J.; Nakamura, M. T.; Cho, H. P.; Clarke, S. D. Sterol regulatory element binding protein-1 expression is suppressed by dietary polyunsaturated fatty acids. A mechanism for the coordinate suppression of lipogenic genes by polyunsaturated fats. *J. Biol. Chem*. **1999**, *274*, 23577–23583.
(27) Piperova, L. S.; Teter, B. B.; Bruckental, I.; Sampugna, J.; Mills, S. E.; Yurawecz, M. P.; Fritsche, J.; Ku, K.; Erdman, R. A. Mammary lipogenic enzyme activity, *trans* fatty acids and conjugated linoleic acids are altered in lactating dairy cows fed a milk fat-depressing diet. *J. Nutr*. **2000**, *130*, 2568–2574.
(28) Corl, B. A.; Oliver, S. A. M.; Lin, X.; Oliver, W. T.; Ma, Y.; Harrell, R. J.; Odle, J. Conjugated linoleic acid reduces body fat accretion and lipogenic gene expression in neonatal pigs fed low- or high-fat formulas. *J. Nutr*. **2008**, *138*, 449–454.
(29) Takahashi, Y.; Kushiro, M.; Shinohara, K.; Ide, T. Activity and mRNA levels of enzymes involved in hepatic fatty acid synthesis and oxidation in mice fed conjugated linoleic acid. *Biochim. Biophys. Acta* **2003**, *1631*, 265–273.
(30) Ferramosca, A.; Savy, V.; Conte, L.; Colombo, S.; Einerhand, A. W. C.; Zara, V. Conjugated linoleic acid and hepatic lipogenesis in mouse: role of the mitochondrial citrate carrier. *J. Lipid Res*. **2006**, *47*, 1994–2003.
(31) Eder, K.; Kirchqessner, M. The effect of dietary vitamin E supply and a moderately oxidized oil on activities of hepatic lipogenic enzymes in rats. *Lipids* **1998**, *33*, 277–283.
(32) Porsgaard, T.; Xuebing, X.; Huiling, M. The form of dietary conjugated linoleic acid does not influence plasma and liver triacylglycerol concentrations in Syrian golden hamsters. *J. Nutr*. **2006**, *136*, 2201–2206.
(33) Terpstra, A. H. M.; Javadi, M.; Beynen, A. C.; Kocsis, S.; Lankhorst, A. E.; Lemmens, A. G.; Mohede, I. C. M. Dietary conjugated linoleic acids as free fatty acids and triacylglycerols similarly affect body composition and energy balance in mice. *J. Nutr*. **2003**, *133*, 3181–3186.

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