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Research Article

# The Effect of Dietary Oils in Prevention of Conjugated Linoleic Acid-Induced Embryo Mortality in Fertile Eggs from Artificially Inseminated Chickens

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**Abstract:** Dietary conjugated linoleic acid (CLA) decreases the ratio of unsaturated fatty acids/saturated fatty acids (UFA/SFA) in yolk and induces embryo mortality in fertile chicken eggs. The objective of this study was to determine if oils rich in UFA would prevent CLA-induced changes in the yolk fatty acid composition and embryo mortality in the fertile eggs. Each diet supplemented with 0.5% corn oil (Group A), 0.5% CLA (Group B), 0.5% CLA plus 10% canola oil (Group C), 0.5% CLA plus 10% olive oil (Group D) or 0.5% CLA plus 10% corn oil (Group E) was fed to 12 laying hens per treatment. Hens were artificially inseminated weekly. Eggs were collected and incubated daily. Three eggs from each group were obtained for fatty acid analysis. After 6 days, Group B had 100% embryo mortality, whereas overall embryo mortality (%) in groups A, C, D and E was 5%, 7%, 4%, and 3%, respectively. The levels of CLA (%) in the yolks from groups A, B, C, D, and E were 0.05%, 2.67%, 1.05%, 0.93%, and 1.25%, respectively. Yolks from Group B had 3.8- and 3.7-fold decreases in the ratios of 16:1(n-7)/16:0 and 18:1(n-9)/18:0, respectively. Adding canola or olive oil to the CLA diet prevented the CLA-induced increase in 16:0 and 18:0 levels and decrease in 18:1(n-9) level. As yolk from the Group B had a higher level of SFA, egg yolk from the groups C, D, and E had a lower level of SFA compared to Group A. While egg yolk from the Group D had increased level of monounsaturated fatty acids (MUFA), the egg yolk from the Group E had a decreased level of MUFA. Dietary CLA decreased the levels of MUFA and UFA significantly ($P < 0.05$). However, adding oils to the CLA diet (Group C, D, or E) restored the level of UFA in the egg yolk. This study shows that oils rich in UFA prevent CLA-induced embryo mortality. This study also suggests that the adverse effects of CLA may be due to an increased ratio of SFA to UFA.

**Key Words:** Conjugated linoleic acid, fatty acids, embryo mortality, laying hens

## Tavuklarda Konjuge Linoleik Asit Beslemesine Bağlı Olarak Oluşan Embryo Ölümlerinin Önlenmesinde Diyetsel Yağların Önemi

**Özet:** Diyetsel konjuge linoleik asit yumurtanın UFA/SFA oranını düşürmekte ve tavuk embryolarında ölümlere neden olmaktadır. Bu çalışmada doymamış yağ asitleri bakımından zengin yağların yumurtanın yağ asiti komposizyonunda CLA'e bağlı olarak oluşan değişiklikleri ve dolayısıyla da embryo ölümlerini önleyip önlemediği araştırılmıştır. Yumurta tavukları (12 adet/grup), % 0,5 mısır yağı (A grubu), % 0,5 CLA (B grubu), % 0,5 CLA+ % 10 kanola yağı (C grubu), % 0,5 CLA+ % 10 zeytin yağı (D grubu) veya % 0,5 CLA + % 10 mısır yağı (E Grubu) eklenen diyetlerle 16 gün boyunca beslenmiştir. Tavukların haftalık suni tohumlamaya tabi tutulmasından sonra toplanan yumurtalar günlük olarak inkübe edilmiştir. Yağ asidi analizi için her gruptan üçer yumurta seçilmiştir. Altıncı gün sonunda B grubunda % 100 embryo ölümleri görülmesine rağmen; A, C, D ve E gruplarındaki embryo ölümleri sırasıyla % 5, 7, 4 ve 3 olarak belirlenmiştir. A, B, C, D ve E gruplarından elde edilen yumurta sarılarında CLA miktarı % 0,05, 2,67, 1,05, 0,93 ve 1,25 olarak belirlenmiştir. A grubuna kıyasla B grubundan elde edilen yumurta sarılarının 16:1(n-7)/16:0 ve 18:1(n-9)/18:0 oranlarında sırasıyla 3,8 ve 3,7 kat azalma gözlenmiştir. CLA içeren diyetlere kanola veya zeytin yağının eklenmesi 16:0 ve 18:0 seviyelerinde görülen artışı ve 18:1(n-9) seviyesinde oluşan azalmayı önlemiştir. B grubu yumurta sarısı, daha yüksek oranda doymuş yağ asiti (SFA) miktarına sahipken, C, D, ve E grubundan elde edilen yumurta sarıları, daha düşük oranda SFA miktarına sahip olmuşlardır. Diyetsel CLA'in kullanımı MUFA ve UFA seviyelerini önemli bir şekilde düşürmüştür. Ancak, CLA içeren diyetlere eklenen sıvı yağlar yumurta sarısındaki UFA seviyelerini restore ederek CLA'in neden olduğu embryo ölümlerini önlemiştir. Ayrıca, bu çalışma diyetsel CLA'nin yan etkilerinin artan SFA/UFA oranına bağlı olabileceğini göstermektedir.

**Anahtar Sözcükler:** Konjuge linoleik asit, yağ asitleri, embryo ölümleri, yumurta tavukları

## Introduction

Conjugated linoleic acid (CLA) is a term used to describe positional and geometrical isomers of linoleic acid (18:2, n-6). In biomedical studies conducted in animal models, several biological activities of CLA were identified, including anti-carcinogenic activity, fat-

reducing effect, immune enhancing activity and possible antiatherogenic properties (1). Consumption of CLA by humans may elicit those favorable health benefits. In a previous study conducted in overweight and obese humans, dietary CLA was shown to reduce body fat mass (2). It was reported that the t-10, c-12 CLA isomer might be the bioactive isomer of CLA to influence the body weight changes observed in subjects with type 2 diabetes mellitus (3).

Major dietary sources of CLA in the human's diet are mainly dairy products and meat from ruminant animals (4). However, the levels of CLA found in those products are not sufficient to obtain these favorable effects of CLA. It was estimated that in order to obtain those health benefits, a 70-kg human subject would have to consume about 1.5 to 3 g of CLA daily (5). There is considerable interest in enriching animal products (egg and dairy products) by supplementing animal feed with CLA (6,7).

Chicken eggs normally contain little or no CLA. It was reported that eggs could be enriched in CLA to as high as 11% by feeding 5% CLA (8). Another study showed that an egg from a chicken fed a diet containing 5% CLA could be enriched about 15% with CLA (9). It was reported that a 60-g egg from laying hens fed a diet containing 5% CLA would provide over one-third of the CLA recommendation (daily 3 g CLA) for an adult human (9). The ingestion of CLA by laying hens resulted not only in incorporation of CLA isomers into egg yolk, but also increased the ratio of saturated fatty acids (SFA) to unsaturated fatty acids (UFA) (8,10). Therefore, CLA in a low-fat diet caused a higher level of embryo mortality in fertile eggs and undesirable color changes in egg yolk and albumen by modifying fatty acid composition of the egg yolk (10,11). For this reason, it is not possible to simply use CLA in laying hen diet and obtain an egg enriched with CLA. Previously, it was shown that CLA-induced embryo mortality in fertile eggs could be prevented by feeding a diet containing olive oil (rich in 18:1, n-9) (10).

Since dietary CLA decreases the ratio of UFA to SFA of egg yolks, the objective of this study was to determine if CLA-induced embryo mortality observed in fertile eggs could be prevented by feeding oils rich in UFA.

## Materials and Methods

Twenty five-week old Single Comb White Leghorn (SCWL) laying hens were randomly distributed into 5 groups of 12 hens each, maintained in individual laying cages. They were assigned to diets containing 0.5% corn oil (Group A), 0.5% CLA (Group B), 0.5% CLA plus 10% canola oil (Group C) or 0.5% CLA plus 10% olive oil (Group D), or 0.5% CLA plus 10% corn oil (Group E) for 16 days. Table 1 represents the dietary treatments. The source of commercial CLA contained 90 % of CLA, Natural Lipids Ltd. AS., Hovdebygda, Norway, and consisted of 41.07% c-9, t-11 and t-9, c-11-CLA; 43.88% t-10, c-12-CLA; 1.69% c-9, c-11-CLA; 1.19% c-10, c-12-CLA; 1.89% t-9, t-11 and t-10, t-12-CLA. Other fatty acids in CLA-90 were 0.87% palmitate, 0.26% stearate, 6.17% oleate, 0.29% linoleate, and 2.69% unknown. The level of CLA used in this study was selected since previous results demonstrated that low-fat diets containing 0.5% CLA induced 100% embryo mortality in the fertile eggs within 7 days by causing significant changes in egg yolk fatty acid composition (10). Wheat middlings were used as a diluent to maintain isocaloric diets in the presence of high levels of oils (10% vegetable oil). Previous work demonstrated that feeding hens up to 89% wheat middlings had no adverse effects on egg quality as was shown by feeding CLA (12). The experimental diets were prepared every week. Dietary oils were mixed and homogenized completely by using a mixer for 5 minutes. Laying hens were exposed to a 16 h light: 8 h dark daily lighting schedule and given free access to water and feed for the duration of the study. After the 16-day feeding period, all laying hens were placed on the control diet (Group A) for 10 days. Laying hens were artificially inseminated weekly with 0.05 ml of pooled semen collected from New Hampshire roosters immediately prior to insemination. Eggs were collected and incubated daily at 37 °C and 85% relative humidity in an incubator. Eggs were candled weekly to detect fertility and embryo death. Hatchability and embryo mortality were computed as a percentage of total fertile eggs on each day.

### Fatty Acid Analysis of Egg Yolk

Eggs (3 per treatment) were obtained for fatty acid analysis on the 7$^{th}$ day of feeding experimental diets. Lipids were extracted from egg yolks with chloroform:methanol (2:1 v/v) (13). Fatty acid methyl esters (FAME) were prepared by reaction with 4% HCl in methanol for 20 min at 60 °C. Gas chromatography (GC) was used to determine the fatty acid composition of egg

Table 1. Composition of experimental diets[1]

| Ingredient | Group A | Group B | Group C | Group D | Group E |
|---|---|---|---|---|---|
| | | | g/100 g diet | | |
| Corn | 67.99 | 67.99 | 22.09 | 22.09 | 22.09 |
| Wheat middlings | 0 | 0 | 39.85 | 39.85 | 39.85 |
| Corn gluten meal | 0 | 0 | 4.62 | 4.62 | 4.62 |
| Soybean meal (44% CP[2]) | 20.39 | 20.39 | 12.24 | 12.24 | 12.24 |
| Calcium carbonate | 8.34 | 8.34 | 8.66 | 8.66 | 8.66 |
| Dicalcium phosphate | 1.21 | 1.21 | 0.45 | 0.45 | 0.45 |
| DL-Methionine | 0.07 | 0.07 | 0.09 | 0.09 | 0.09 |
| Corn oil | 0.5 | 0 | 0 | 0 | 10 |
| CLA | 0 | 0.5 | 0.5 | 0.5 | 0.5 |
| Canola oil | 0 | 0 | 10 | 0 | 0 |
| Olive oil | 0 | 0 | 0 | 10 | 0 |
| Salt | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Premix[3] | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |

[1] Diets were isonitrogenous and isocaloric and calculated to contain 15% CP and 2800 kcal/kg ME. To maintain isocaloric diets in the presence of high levels of vegetable oils, wheat middlings were used as a diluent.

[2] CP= crude protein

[3] Supplied per kg of diet: vitamin A, 10,000 IU; vitamin $D_3$, 9790 IU; vitamin E, 121 IU; $B_{12}$, 20 µg; riboflavin, 4.4 mg; calcium panthothenate, 40 mg; niacin, 22 mg; choline, 840 mg; biotin, 30 µg; thiamin, 4 mg; zinc sulfate, 60 mg; manganese oxide, 60 mg.

yolks. Briefly, a Hewlett-Packard 5890 series II GC was fitted with a flame-ionization detector and 3396A integrator. A supelcovax-10 fused silica capillary column (60 m X 0.32 mm i.d., 0.25 µm film thickness) was used. Oven temperature was programmed from 50 to 200 °C, increased 20 °C per min, held for 50 min, increased 10 °C per min to 230 °C, and held for 20 min. Heptadecanoic acid (Sigma Chemical Co., St Louis, MO, USA) was used as an internal standard. The FAME were identified by comparison of retention times with methylated fatty acid standards (Sigma Chemical Co., and Nu-Chek Prep., Elysian, MN, USA) and expressed as percentage of total FAME (4).

### Statistical analysis

In the statistical analysis of hatchability, data for 16 days were reported for each dietary treatment. To compare the hatchability of dietary treatments over time, the time in the study was divided into 2 periods (1: days 1-6; 2: days 7-16). For each period, regression lines were fitted for each dietary treatment. Therefore, the difference between any treatments was examined by t-test on 2 parameters (slope and intercept). Different slope or intercept indicated that the 2 regression lines were different (Figure). Results were considered significant at the level of $P < 0.05$. Statistical analysis of fatty acid content was performed by one-way ANOVA using the General Linear Models procedure (14). Multiple and pair-wise comparisons were made through Fisher's least significant difference (LSD) procedure in the cases in which the overall effect was significant ($P < 0.05$).

## Results

The Figure represents the effects of diet containing CLA and high levels of oils on hatchability of fertile eggs in chickens. CLA in a low-fat diet (Group B) caused 100% embryo mortality in the fertile eggs after the 6 days of feeding. Adding 10% oils (canola oil, olive oil or corn oil) completely prevented CLA-induced embryo mortality in the fertile eggs. Overall hatchability (%) of fertile eggs

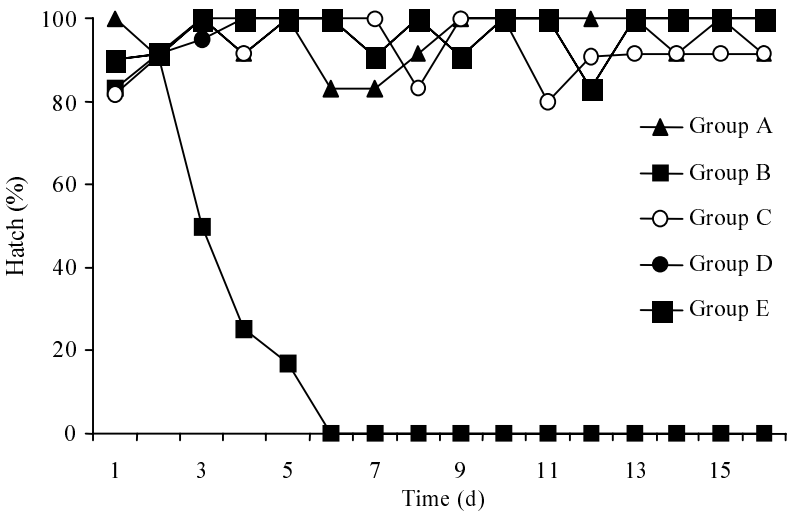


Figure. Hatchability (%) of fertile eggs from chickens fed diets containing conjugated linoleic acid with and without additional vegetable oil.
Each point in the figure represents hatchability of 7-12 fertile eggs. Embryo mortality in the CLA group was significantly greater than that of the control group. In the first period of the experiment, only the slope of the CLA group was significantly different from that of the control group ($P < 0.001$). In the second period, both the intercept and slope of the CLA group were significantly different from that of the control group ($P < 0.001$ and $P < 0.01$, respectively).

from the groups A, C, D, and E did not vary, 95%, 93%, 96%, and 97%, respectively (Figure).

Feeding CLA in low-fat diet (Group B) increased the level of SFA and decreased the levels of MUFA and UFA in the egg yolk (Table 2). Egg yolk from Group B had significantly greater levels of 14:0 than in eggs from the controls ($P < 0.05$). However, adding oils to the CLA diet (Group C, D, or E) caused significant decreases in the levels of 14:0 and 16:0 compared to the controls ($P < 0.05$). Egg yolk from Group B had higher levels of 16:0 and 18:0 and lower levels of 18:1(n-9) compared to the other groups ($P < 0.05$). Adding 10% olive oil or canola oil diet prevented CLA-induced decreases in the level of 18:1(n-9). However, the diet supplemented with 10% corn oil (Group E) did not prevent CLA-induced decreases in the level of 18:1(n-9), but caused a significant decrease in 18:1(n-9) compared to the controls ($P < 0.05$). The level of 18:2(n-6) in egg yolk from Group E increased significantly ($P < 0.05$). Egg yolk from Groups C and E had an increased level of 18:3(n-3) compared to the other groups ($P < 0.05$). Similar to the level of 18:2(n-6), the level of 20:4(n-6) significantly increased in the egg yolk from Group E. However, the level of 20:4(n-6) in the egg yolk from Group B was significantly lower than that in Groups A, D and E ($P < 0.05$). Adding 10% oil (Group C, D or E) caused a significant decrease in the level of CLA accumulation in the egg yolk compared to Group B. Relative amounts of CLA (% of FAME) were 0.05%, 2.67%, 1.05%, 0.93%, and 1.25%, in Groups A, B, C, D and E, respectively. The levels of c-9, t-11 CLA (main isomer of CLA) and t-10, c-12 CLA isomers in the egg yolk from Group B were significantly higher than those in Groups A, C, D, and E ($P < 0.05$). In addition, egg yolk from Group B had a significantly higher level of SFA than that from the other groups. Adding oils to the diet in the presence of CLA (Group C, D, or E) caused a significant decrease in the level of SFA in egg yolk. Use of 10% canola oil or olive oil (Group C or D) restored the levels of MUFA in the egg yolk compared to Group B. Adding oils to the CLA diet (Group C, D, or E) also restored the levels of UFA in the egg yolk. However, adding 10% corn oil (Group E) did not restore the level of MUFA in the egg yolk, but caused a significantly higher level of PUFA (2.6-fold increase) in the egg yolk.

Table 2. Effect of experimental diets containing conjugated linoleic acid with added canola oil, olive oil or corn oil on select fatty acid composition of egg yolk lipid in chickens[1]

| Fatty Acids | Dietary Treatments[2] | | | | |
|---|---|---|---|---|---|
| | Group A | Group B | Group C | Group D | Group E |
| | % FAME | | | | |
| 14:0 | 0.52 ± 0.0[b] | 0.72 ± 0.03[a] | 0.28 ± 0.01[c] | 0.27 ± 0.01[c] | 0.28 ± 0.0[c] |
| 16:0 | 28.58 ± 0.07[b] | 34.72 ± 0.04[a] | 21.62 ± 0.53[d] | 23.85 ± 0.31[c] | 23.53 ± 0.05[c] |
| 16:1(n-7) | 4.15 ± 0.01[a] | 1.27 ± 0.06[b] | 0.60 ± 0.03[c] | 0.57 ± 0.01[c] | 0.49 ± 0.04[c] |
| 18:0 | 9.26 ± 0.02[b] | 19.23 ± 0.03[a] | 9.61 ± 0.38[b] | 10.01 ± 0.84[b] | 11.00 ± 0.21[b] |
| 18:1(n-9) | 36.35 ± 0.09[c] | 20.58 ± 0.01e | 38.57 ± 0.67[b] | 43.36 ± 0.04[a] | 23.83 ± 0.42[d] |
| 18:2(n-6) | 10.66 ± 0.04[c] | 11.91 ± 0.08[c] | 16.18 ± 0.21[b] | 11.77 ± 0.64[c] | 29.98 ± 0.58[a] |
| 18:3(n-3) | 0.21 ± 0.0[d] | 0.29 ± 0.01[c] | 1.65 ± 0.03[a] | 0.26 ± 0.01[cd] | 0.41 ± 0.02[b] |
| 20:4(n-6) | 1.56 ± 0.01[b] | 1.32 ± 0.01[c] | 1.46 ± 0.02[bc] | 1.56 ± 0.05[b] | 1.87 ± 0.08[a] |
| c-9, t-11 CLA | 0.05 ± 0.05[d] | 1.55 ± 0.02[a] | 0.84 ± 0.01[bc] | 0.71 ± 0.03[c] | 1.00 ± 0.05[b] |
| t-10, c-12 CLA | nd | 0.67 ± 0.02[a] | 0.21 ± 0.01[b] | 0.22 ± 0.01[b] | 0.25 ± 0.02[b] |
| ΣCLA | 0.05 ± 0.05[d] | 2.67 ± 0.04[a] | 1.05 ± 0.05[bc] | 0.93 ± 0.08[c] | 1.25 ± 0.11[b] |
| ΣSFA | 38.36 ± 0.09[b] | 54.66 ± 0.11[a] | 31.52 ± 0.92[d] | 34.14 ± 0.73[c] | 34.81 ± 0.26[c] |
| ΣMUFA | 40.50 ± 0.10[b] | 21.85 ± 0.07[d] | 39.17 ± 0.67[b] | 43.94 ± 0.05[a] | 24.32 ± 0.46[c] |
| ΣPUFA | 12.43 ± 0.06[c] | 13.52 ± 0.07[c] | 19.30 ± 0.19[b] | 13.60 ± 0.70[c] | 32.27 ± 0.52[a] |
| ΣUFA | 52.93 ± 0.08[b] | 35.37 ± 0.09[c] | 58.47 ± 0.65[a] | 57.54 ± 0.86[a] | 56.59 ± 0.96[a] |

[1]Values are means ± SD expressed as percentage total fatty acids. n = 3 yolk samples per treatment and means with different superscripts within a row are significantly different ($P < 0.05$).

ΣCLA: total conjugated linoleic acid; ΣSFA: total saturated fatty acids; ΣMUFA: total monounsaturated fatty acids; ΣPUFA: total polyunsaturated fatty acids; ΣUFA: total unsaturated fatty acids; ΣUFA and ΣPUFA do not include CLA-isomers

nd: not detectable

## Discussion

Yolk fat as a source of energy and essential nutrients has a crucial role in avian embryo development (15). Significant alterations in yolk fatty acid composition can have drastic effects on embryonic survival. Dietary CLA was shown to influence the fatty acid composition of yolk by inhibiting stearoyl-CoA desaturase, an enzyme that catalyzes the insertion of a double bond between the C-9 and C-10 atoms of either 16:0 or 18:0 in the formation of 16:1(n-7) and 18:1(n-9), respectively (16), and induce embryo mortality in chickens (10) and Japanese quail (11). In the previous study, it was shown that dietary CLA (at the level of 0.5%) increased the level of SFA (mainly 16:0 and 18:0) and decreased the level of MUFA (mainly 16:1, n-7 and 18:1, n-9) and caused 100% embryo mortality in 6 days of feeding (10). In the same study, adding 10% olive oil, rich in 18:1(n-9), restored the level of MUFA and completely prevented CLA-induced embryo mortality in fertile chicken eggs. Similarly, in the present study, CLA in the low-fat diet (Group B) caused complete embryo mortality after 6 days of the study. And addition of oils high in MUFA or PUFA also prevented CLA-induced embryo mortality of fertile eggs (Figure). Egg yolk lipids exhibit a fatty acid pattern resembling that of the dietary oils. Hence, in the present study canola oil and olive oil (rich in 18:1, n-9) enriched the egg yolks with 18:1(n-9), whereas corn oil rich in 18:2(n-6) caused a higher level of 18:2(n-6) in the yolks compared to the controls.

CLA in the low-fat diet caused significant alterations in the yolk fatty acid composition and increased embryo

mortality in fertile eggs. Adding oils (high in MUFA or PUFA) to the CLA diet (Group C, D or E) completely prevented CLA-induced embryo mortality by restoring the UFA level of the egg yolk. In the present study, addition of oils (Group C, D, or E) lowered the levels of c-9, t-11 and t-10, c-12 CLA isomers significantly in the egg yolk compared to Group B. However, it was reported that egg CLA was not directly toxic for the developing chick embryo (17). This study suggests that the adverse effects of CLA on the hatchability of fertile chicken egg may be due to the increased level of SFA to UFA.

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