Isomers of Conjugated Linoleic Acid (CLA) Are Incorporated into Egg Yolk Lipids by CLA-Fed Laying Hens

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ABSTRACT This study was designed to determine the amount of conjugated linoleic acid (CLA) incorporated into egg lipids after dietary CLA supplementation. Single Comb White Leghorn laying hens (n = 40; 28 wk old) were randomly assigned to four treatments of varying CLA levels (0, 0.01, 0.5 and 1 g CLA/kg diet). Eggs were collected daily for 36 d. Feed consumption and body weight were monitored. CLA content of egg yolk lipid was analyzed by gas-liquid chromatography. Birds fed 0.5 and 1.0 g CLA/kg feed had significantly more CLA in the egg yolk lipid vs. control and 0.01 g CLA/kg diet groups after 7 d (P < 0.0004). Incorporation of CLA into egg lipid was highest on d 24 and 36. CLA enrichment in egg lipid in the 1.0 g CLA/kg diet group was similar to that in ruminant animal food products, ~3 mg CLA/g fat. J. Nutr. 130: 2002–2005, 2000.

KEY WORDS: • conjugated linoleic acid • fatty acid • laying hens • lipid • egg

Isomers of conjugated linoleic acid (CLA) have anticancer activity (Belury 1995, reviewed by Ip 1994, Scimeca et al. 1994), immune-enhancing qualities (Cook et al. 1993, Miller et al. 1994), weight-reducing effects (Belury and Kempa-steczko 1997, Scimeca et al. 1994) and possible antiatherogenic properties (Lee et al. 1994, Nicolosi et al. 1997) in animals at levels below 1% of the total energy in the diet (Ip et al. 1994). The e9, t11–18:2 isomer has been implicated as the biologically active form. CLA is found predominantly in food items produced from ruminant animals. The e9, t11–18:2 isomer is the predominant form found in foods, comprising >75% of all of the CLA isomers (Chin et al. 1992). Daily intake of CLA is not well documented but has been estimated to be under several hundred mg/d (Ens et al., unpublished data, Fritsche and Steinhart 1998, Ip et al. 1994). Animal data indicate that ~3.0 g/d of CLA may be necessary for beneficial effects in humans (Ip et al. 1994). Reevaluation of these data on the basis of total dietary energy consumption suggests that 600 mg CLA/d may have anticancer effects (Ens et al., unpublished data).

It is possible to change the lipid composition of food products, such as eggs, by modifying the diet of the laying hens (Cruickshank 1934). The development of CLA-enriched foods could have implications in the poultry industry by improving immunity and health, increasing growth and improving feed efficiency (Chin et al. 1994, Miller et al. 1994, Park et al. 1997). CLA fortification could contribute to diet-based cancer prevention in human populations (Hargis et al. 1991, Jiang et al. 1993). Thus, the objective of this study was to develop a feeding regimen to produce CLA-rich eggs. The short- and long-term effect of CLA supplementation for 36 d on the CLA content in eggs, the CLA content in the body fat of Shaver 2000 laying hens and on the weight of the hens were determined.

MATERIALS AND METHODS

Diets. Shaver 2000 Single Comb White Leghorn pullets (n = 40) were reared in floor pens to 18 wk of age. The rations were provided as follows: chick starter from 0 to 6 wk; grower ration 1 from 6 to 16 wk; and layer ration from 16 to 29 wk. At 18 wk, hens were moved to individual laying cages. At 29 wk, hens were fed a modified layer ration until the end of lay. The four diets consisted of a control diet (layer ration containing no CLA), a low CLA diet containing 0.01 g CLA/kg diet (0.001% wt/wt, 0.04% total added fat), a medium diet with 0.5 g CLA/kg feed (0.05% wt/wt, 1.8% total added fat), and a high CLA diet having 1.0 g CLA/kg feed (0.1% wt/wt, 3.7% added fat). Feed and water were consumed ad libitum from individual containers inaccessible to neighboring birds. Ten birds completed each diet treatment.

Experimental design. Pullets were reared in light-tight floor pens with a bird density of 15.24 cm2/bird until 10 wk, then 60.96 cm2/bird until 16 wk. Chicks were subjected to a photoschedule of 23 h light (L) to 1 h dark (D) (23L:1D), which was reduced to 8L:16D at 4 d and maintained until 18 wk of age. Beak trimming was performed between 5 and 8 d of age. At 18 wk of age, pullets were moved to individual laying cages and fed a standard layer diet. Once in laying cages, the photoschedule was increased to 11L:13D, and was increased by 0.5 h of light/wk until it reached 14L:10D. At 29 wk, 40% of pullets were weighed and assigned to one of the following four treatment groups: a control group (standard layer diet), CLA-enriched diets of 0.01, 0.5 or 1.0 g CLA/kg diet. Pullets were fed these diets for 36 d and eggs were collected on d 1–12, 24 and 36. Individual feed intakes were calculated weekly. Intakes were calculated by feed weigh-back, through recording the feed given, and subtracting the initial starting weight and the end weight to give the total feed consumed. Body weight was measured biweekly. After 36 d, sixty hens from each group of ten were returned to the general laying population, and the remaining hens from each group were fed their respective diets until they reached the age of 68 wk. Feed intakes and body weight measures were taken every 4 wk until the end of the lay. All birds survived until 68 wk of age. On the afternoon before being killed, the birds were deprived of food overnight (12–20 h) to permit gut content clearance. All experimental procedures performed on live birds were approved by the University of Alberta Faculty of Agriculture, Forestry and Home Economics Animal Policy and Welfare Committee.

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**Synthesis of CLA.** CLA was obtained as described by Ma et al. (1999). Linoleic acid was purified from safflower oil and isomerized using a modified method described by Chin et al. (1992). The CLA obtained had a purity of 95%. The majority of the isomers present were 9c,11t-18:2 and 10t,12c-18:2 in approximately equal amounts, which accounts for 94.6% of total CLA content.

**Experimental diets.** Layer rations3 (University of Alberta Edmonton Research Station) were prepared with the exclusion of canola oil from the mixture. Canola oil (Country Harvest 100% Pure Canola oil, Lucerne Foods, Vancouver, Canada) was purchased from commercial sources. Diets were prepared individually in quantities of 35 kg, estimated to last ~30 d, and diets were always prepared in a consistent order starting with the control diet, 0.01 g CLA/kg feed (low), 0.5 g CLA/kg feed (medium) and 1.0 g CLA/kg feed (high) to avoid CLA contamination in feeds with a lower CLA concentration. The amount of canola oil that was required for a standard layer ration (control diet) of 35 kg of feed is 959 g (27.4 g/100 g). The amount of CLA required for each concentration was added (factoring in the 95% purity of CLA) to canola oil until the desired weight of 959 g was achieved. The oil was mixed for 10 min to ensure a uniform blend. The diet was mixed and stored at 4°C.

**Egg production.** Individual daily egg records were kept for the hens until 68 wk. Egg production was expressed as average hen-day production, calculated from the total eggs divided by the number of days. Hen-day production was calculated for the entire study. Egg and yolk weights were measured for eggs collected on d 1–12, 24 and 36. Yolks were separated and were stored in 15-mL vials at −5°C until extraction.

**Fatty acid analysis of yolk lipids.** Egg yolk lipid was extracted by the method of Folch et al. (1957). Yolk lipid (5 mg) and 25 μL 0.05 mol/L NaOH/methanol. Samples were heated for 1 h at 110°C in a sand bath, then cooled. Samples were methylated at room temperature as described by Werner et al. (1992). Fat content was expressed relative to dry lipid weight. All gas-liquid chromatography (GLC) analysis was carried out with a Varian 6000 gas chromatograph (Georgetown, Canada) utilizing a Varian Star Chromatography Workstation (version 4.0). CLA content was quantified in duplicate using a SP-2560 fused silica capillary column (100 m × 0.25 mm i.d., 0.2 μm film thickness; Supelco, Bellefonte, PA) as described by Ma et al. (1999).

**Statistical analysis.** Fatty acid profiles were derived from GLC analysis using values obtained with the 19:0 internal standard as a reference. CLA was measured quantitatively. A two-way ANOVA was used to compare variables between treatment groups and days. Orthogonal comparisons were used to determine significant differences in egg yolk lipids. Values are means ± SEM; n = 10. Means without a common letter differ significantly (P < 0.05).

**RESULTS**

**Egg weights, yolk weights and egg production.** No significant differences were observed in egg (data not shown) or

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**FIGURE 1** Change in yolk weight in laying hens fed a modified layer ration providing fed diets containing 0, 0.01, 0.5 or 1.0 g conjugated linoleic acid/kg of diet for 36 d beginning at 29 wk of age. There was no effect of diet treatment on yolk weights; therefore all diet treatments were combined to illustrate this data. Values are means ± SEM, n = 10. Means without a common letter differ significantly (P < 0.05).

**FIGURE 2** Conjugated linoleic acid incorporation (CLA) into yolk lipids of laying hens fed diets containing 0, 0.01, 0.5 or 1.0 g CLA/kg of diet beginning at 29 wk of age. CLA incorporation into egg yolk lipid was determined. Values are means ± SEM, n = 10. a,b,cIndicates a significant increase from d 0 values for the medium group (P < 0.0001). dIndicates a significant increase from d 7–12 for the medium group (P < 0.001). eIndicates a significant increase from d 7–12 for the high group (P < 0.01). fIndicates a significant increase from all other days (P < 0.003). Means for a day without a common letter differ (P < 0.05).

**FIGURE 3** Conjugated linoleic acid (CLA) isomer incorporation into yolk lipids for hens fed a modified layer ration providing fed diets containing 0, 0.01, 0.5 or 1.0 g CLA/kg of diet for 36 d beginning at 29 wk of age. Values are means ± SEM, n = 10. a,b,cAbove a column indicates a significant difference in c9,t11 between groups (P < 0.05). dAbove a column indicates a significant difference between isomers within a group (P < 0.05). Means without a common letter differ (P < 0.05).
yolk weights (Fig. 1) among groups. Egg production, expressed relative to hen-day production, was significantly influenced by diet ($P < 0.05$), with the control and low CLA group having the highest laying rates throughout the study, followed by the medium and then the high CLA-fed groups (95.26, 94.05, 91.62 and 88.84%, respectively). **CLA incorporation into yolk lipid.** To assess the relative and absolute abundance of yolk CLA and to enable comparisons with other studies, levels were expressed relative to fat content (Fig. 2). The CLA level for all groups at d 0 was $\sim 2\ \mu$mol CLA/g lipid. After 36 d, egg yolk CLA in the three CLA-fed groups was greater than in the control group. Within 1 wk of feeding, the medium and the high CLA groups had a 68–73% increase in egg yolk CLA content compared with the d 0 value ($P < 0.0004$). Significant differences in yolk CLA content between the medium and high CLA groups became evident between d 9 and 12 of feeding, with the high CLA-fed group having more yolk CLA ($P < 0.0001$). In the medium CLA-fed group, egg yolk CLA on d 24 was significantly higher than on d 12 ($P < 0.0001$), but was not different from d 36. In contrast, egg yolk CLA in the high CLA-fed group increased significantly between d 12 and 24 ($P < 0.01$), and again between d 24 and 36 ($P < 0.003$). After 36 d, the $c_9,t_11$ isomer of CLA was preferentially incorporated into the yolk lipid compared with the $t_10,c_{12}$ CLA isomer ($P < 0.0001$) in all groups (Fig. 3). The ratio of $c_9,t_11$ CLA to $t_10,c_{12}$ CLA in the yolk lipid of the high CLA diet group was 4:1 and in the medium CLA diet, 30:1. **Body weight changes.** At the end of the study, the birds fed the control and low CLA diets did not differ in body weight (1.81 $\pm$ 0.025 and 1.82 $\pm$ 0.042 kg, respectively), but birds of both groups weighed significantly more than the birds fed the medium and high CLA diets by wk 5 (1.64 $\pm$ 0.013 and 1.72 $\pm$ 0.019 kg, respectively; $P < 0.035$) (Fig. 4). Birds fed the medium CLA diet were significantly smaller than all other birds beginning at wk 24 ($P < 0.0007$). **Feed intake.** No differences in relative feed intakes (g feed/kg body) were found among groups within a given week, except during wk 5 when birds of the medium CLA-fed group (0.05 g CLA/kg) consumed significantly more food ($P < 0.008$) than all other groups (data not shown). Birds of the high and low CLA-fed groups ate $\sim 4$–$7\%$ less ($P < 0.025$) relative to body weight than birds of the control and medium CLA groups over the entire study. **DISCUSSION** High and medium CLA-fed groups had lower egg production rates than those fed 0 and 0.1 mg CLA/kg, indicating that CLA affects reproductive efficiency of the hens. The reason for this finding requires further investigation. The development of CLA-enriched eggs may have consumer appeal. The diet-induced increase in egg yolk CLA content was dose dependent; in the medium and high CLA-fed groups (0.5 and 1.0 g CLA/kg), this effect was observed within 1 wk of feeding. Eggs from the medium CLA-fed group reached maximum CLA incorporation after 24 d, whereas the high CLA diet group may not have reached maximum incorporation of CLA into the yolk after 36 d. Thus, it may be possible for even higher levels of CLA to be incorporated when fed at a dietary level of 1.0 g CLA/kg. The amount of CLA that was incorporated after 36 d of feeding the high CLA diet was 3.33 mg (12 $\mu$mol) CLA/kg fat or $\sim 15$ mg of CLA/egg, an amount similar to that in ruminant animal products, such as a glass of milk (Chin et al. 1992). The $c_9,t_1$–$c_9,t_2$ isomeric form of CLA was incorporated preferentially relative to the $t_10,c_{12}$–$c_9,t_2$ isomer, despite being fed in approximately the same proportion in the diet. This agrees with other data showing that the $c_9,t_1$–$c_9,t_2$ isomer is incorporated preferentially to the $t_10,c_{12}$ isomer when fed to rats at approximately the same ratio (Sugano et al. 1997, Winchell et al. 1998). Feeding CLA has been reported to reduce body fat deposits of mice and chickens (Belury and Kempa-steczko 1997, Cook et al. 1993), by $\geq 50\%$ (Park et al. 1997). In this study, body weights of birds of the medium and high CLA-fed groups did not change significantly over the 33-wk period. Birds of the control and low CLA-fed groups exhibited increased weight after 27 wk of feeding, and became statistically heavier than birds of the medium CLA-fed group after 5 wk. The groups fed higher amounts of CLA (0.5 and 1.0 g CLA/kg) did not become heavier as is usually the case in laying hens (Branton et al. 1995, Rothenbacher et al. 1972, Squires and Leeson 1988). **LITERATURE CITED** Belury, M. A. (1995) Conjugated dioinoic linoleate: a polyunsaturated fatty acid with unique chemoprotective properties. Nutr. Rev. 53: 83–89. Belury, M. A. & Kempa-steczko, A. (1997) Conjugated linoleic acid modulates hepatic lipid composition in mice. Lipids 32: 199–204. Branton, S. L., Lott, B. D., Maslin, W. R. & Day, E. J. (1995) Fatty liver-hemorrhagic syndrome observed in commercial layers fed diets containing chelated minerals. Avian Dis. 39: 631–35. Chin, S. F., Storkson, J. M., Albright, K., Cook, M. E. & Pariza, M. W. 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