

Three Hen Strains Fed Photoisomerized *trans,trans* CLA-Rich Soy Oil Exhibit Different Yolk Accumulation Rates and Source-Specific Isomer Deposition

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Abstract Most CLA chicken feeding trials used *cis,trans* (*c,t*) and *trans,cis* (*t,c*) CLA isomers to produce CLA-rich eggs, while reports of *trans,trans* (*t,t*) CLA enrichment in egg yolks are limited. The CLA yolk fatty acid profile changes and the 10–12 days of feeding needed for maximum CLA are well documented, but there is no information describing CLA accumulation during initial feed administration. In addition, no information on CLA accumulation rates in different hen strains is available. The aim of this study was to determine a mathematical model that described yolk CLA accumulation and depletion in three hen strains by using *t,t* CLA-rich soybean oil produced by photoisomerization. Diets of 30-week Leghorns, broilers, and jungle fowl were supplemented with 15 % CLA-rich soy oil for 16 days, and eggs were collected for 32 days. Yolk fatty acid profiles were measured by GC-FID. CLA accumulation and depletion was modeled by both quadratic and piecewise regression analysis. A strong quadratic model was proposed, but it was not as effective as piecewise regression in describing CLA accumulation and depletion. Broiler hen eggs contained the greatest concentration of CLA at 3.2 mol/100 g egg yolk, then jungle fowl at 2.9 mol CLA, and Leghorns at 2.3 mol CLA. The *t,t* CLA isomer levels remained at 55 % of total yolk CLA during CLA feeding. However, *t-10,c-12* (*t,c*) CLA concentration increased slightly during CLA accumulation and was significantly greater than *c-9,t-11* CLA. Jungle fowl

had the smallest increase in yolk saturated fat with CLA yolk accumulation.

Keywords *Trans* · *Trans* conjugated linoleic acid · Egg yolks · Modeling · Accumulation · Depletion · Fatty acid analysis

Abbreviations

CLA	Conjugated linoleic acid
<i>c,t</i>	<i>cis,trans</i>
DHA	Docosahexaenoic acid (22:6n-3)
EPA	Eicosapentaenoic acid (20:5n-3)
FA	Fatty acid
FAME	Fatty acid methyl ester
GC-FID	Gas chromatography-flame ionization detector
HME	Heptadecanoic acid methyl ester
MUFA	Monounsaturated fatty acid
PUFA	Polyunsaturated fatty acid
RBD	Refined, bleached, deodorized
SFA	Saturated fatty acid
<i>t,c</i>	<i>trans,cis</i>
<i>t,t</i>	<i>trans,trans</i>

Introduction

Poultry feeding trials have used *cis,trans* (*c,t*) and *trans,cis* (*t,c*) CLA isomers to obtain CLA-rich eggs [1–7], resulting in up to 11 % CLA in yolk total fatty acids when chickens were fed 5 % CLA [1]. The *c-9, t-11* CLA isomer has been shown to be preferentially incorporated into yolks, even when the isomer concentration in the feed is equal to that of *t-10, c-12* CLA [1, 3, 5]. CLA concentration plateaus and stabilizes after 11 days of CLA administration, corresponding to 10–12 day yolk follicle maturation to full

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ovulation [1–5]. CLA egg enrichments have resulted in increased saturated fat and decreased monounsaturated fat levels in the yolks [1–5]. While changes in yolk fatty acids and the time it takes eggs to reach maximum CLA is well documented, there is no information describing CLA accumulation in those initial 10–12 days of feed administration.

Reports on different hen breeds ability to incorporate CLA in their yolks is limited. One study showed that while both white Leghorns and brown dwarf layer hen breeds incorporated the same CLA amounts in yolk, white Leghorn hens had higher amounts of yolk *c,t* isomers and lower amounts of *t,c* CLA in comparison to brown dwarf hens. In addition, CLA isomer concentrations did not reflect the diets' isomer composition [3, 5]. Broiler hens have been supplemented with CLA, which resulted in 6 % CLA in breast muscle lipids and 10 % CLA in leg muscle lipids, and abdominal fat deposition was significantly reduced [6]. However, the study did not determine yolk CLA concentration.

Recently, *trans,trans* (*t,t*) CLA isomers have shown superior anti-carcinogenic and anti-atherogenic effects in mouse and rat models [8–11]. In a comparative in vitro study, various CLA isomers were compared for the strongest anti-carcinogenic effect on colon cancer cells [9]. The strongest inhibition was demonstrated by *-t-9,t-11* CLA, followed by *t-10,c-12* CLA, *c-9,c-11* CLA, and *c-9,t-11* CLA, respectively. Reports of *t,t* CLA superior anti-carcinogenic activity in MCF-7 human breast cancer cells and rat mammary tumorigenesis have also been published [8, 10]. *Trans,trans* CLA isomers have not been extensively studied due to lack of availability, despite the health benefits of *t,t* CLA that are now being realized [11]. Thus, in a separate study [12], it was determined that CLA enriched phospholipids and triacylglycerols were produced in egg yolks from chickens fed *t,t* CLA-rich soybean oil produced by photoisomerization. This CLA-rich soybean oil was produced by photoisomerization of soy oil linoleic acid, of which 70 % of the CLA isomers are in *t,t* form [13]. *Trans,trans* CLA-rich soybean oil was fed to obese rats and significantly decreased LDL, cholesterol, and liver lipid levels [14].

All previous CLA egg accumulation studies have been conducted with *c,t/t,c* CLA mixtures and, therefore, studies with *t,t* CLA are needed. This can be readily done with CLA-rich soy oil. Furthermore, yolk CLA and individual CLA isomer accumulation as a function of feeding duration, hen breed, or CLA depletion rates after dietary CLA removal has not been reported.

The aim of this study was to determine a statistical model that best described CLA egg yolk accumulation and depletion following feeding *t,t* CLA-rich soybean oil to commercial Leghorns, commercial broilers, and jungle fowl. The specific objectives were to (1) Compare statistical

models that describe CLA accumulation and depletion rates in CLA-rich egg yolks of broilers, Leghorns, and jungle fowl; (2) Compare *t,t* CLA yolk accumulation with that of other CLA isomers from CLA-rich soybean oil across the three hen varieties; and (3) Determine the changes in fatty acid composition in response to *t,t* CLA yolk accumulation and breed.

Materials and Methods

Materials

Refined, bleached and deodorized (RBD) soybean oil for CLA-rich soybean oil production was obtained from Rice-land Foods (Stuttgart, AR, USA). Resublimed iodine crystals were used as a catalyst for CLA-rich soybean oil production (EM Science, Cherry Hill, N.J., USA). A standard commercial feed was obtained from Cobb Poultry Feed Mill (Cobb-Vantress Inc., Siloam Springs, AR, USA). This feeding trial was approved by the Arkansas Institutional Animal Care and Use Committee (Protocol # 13033).

Methods

Model Comparisons Describing CLA Accumulation and Depletion

CLA-Rich Soy Oil Production

A CLA-rich soybean oil containing 16 % CLA was produced by the method of Jain et al. [13]. In summary, RBD soybean oil was combined with 5 % Magnesol (commercial magnesium silicate, wt/wt) and mixed using a stir-bar for 20 min (The Dallas Group of America, Inc., Whitehouse, NJ). In a previous study it was found that Magnesol adsorption removed large quantities of phospholipids and peroxides from soy oil and greatly increased CLA yields [15]. The solution was filtered using Whatman #4 filter paper with aid of a vacuum pump. The oil was then heated to 55 °C and 0.25 % of resublimed iodine was added [13]. The oil was then exposed to UV radiation for 12 h [13]. Table 1 shows the fatty acid concentration of the original RBD soy oil and CLA-rich soy oil.

Dietary Treatments

The feed used for the study was a pelleted commercial corn and soybean meal-based finisher diet provided by Cobb-Vantress (Siloam Springs, AR). Feed was formulated without meat or animal by-products to meet or exceed the minimum NRC (1994) standards for all ingredients. Nutrient

Table 1 Fatty acid composition of refined, bleached, deodorized (RBD) soy oil, and resulting CLA-rich soy oil after 12 h photoisomerization, which resulted in 16 % CLA

Fatty acid	RBD soy oil (g FA/100 g oil)	CLA-rich soy oil (g FA/100 g oil)
Palmitic acid 16:0	12.2 ± 0.05	12.2 ± 0.02
Palmitoleic acid 16:1	0.09 ± 0.01	0.09 ± 0.01
Stearic acid 18:0	4.12 ± 0.04	4.24 ± 0.02
Oleic acid 18:1	25.0 ± 0.05	25.9 ± 0.06
Linoleic acid 18:2	55.0 ± 0.07	38.5 ± 0.05
Linolenic acid 18:3	3.54 ± 0.02	3.59 ± 0.02
<i>cis</i> -9, <i>trans</i> -11 CLA	ND	1.44 ± 0.01
<i>trans</i> -9, <i>cis</i> -11 and <i>cis</i> -10, <i>trans</i> -12 CLA	ND	1.89 ± 0.02
<i>trans</i> -10, <i>cis</i> -12 CLA	ND	1.12 ± 0.01
<i>trans</i> , <i>trans</i> CLA	ND	11.5 ± 0.02
Total CLA	ND	16.0 ± 0.03

ND not detected

Table 2 Percentage composition of the Cobb pelleted commercial feed that was used to prepare experimental diets

Metabolizable energy (kcal/kg)	2761
Crude protein (%)	15.43
Calcium (%)	1.45
Phosphorous (%)	0.43
Sodium (%)	0.15
Linoleic acid (%)	0.15
Amino acids	
Lysine (%)	0.61
Methionine (%)	0.27

The feed used for the study was a pelleted commercial corn and soybean meal-based finisher diet provided by Cobb-Vantress (Siloam Springs, AR). Feed was formulated without meat or animal by-products to meet or exceed minimum NRC (1994) standards for all ingredients

levels of the unmodified feed are shown in Table 2. The control diet was prepared by adding 10 % (wt) RBD soybean oil to the commercial feed. Ten percent CLA-rich soybean oil (% wt) was added to the commercial feed for the CLA experimental diet. Diets were prepared using a Hobart stand mixer (Hobart Legacy HL 200) to adequately mix oil with feed. Fresh feed was produced every three days, and CLA-rich soybean oil was flushed with nitrogen and stored at 4 °C to inhibit lipid oxidation.

Experimental Design

Ten commercial white Cobb broilers, 10 Cobb white Leghorns, and 10 full-colored jungle fowl, all 30-weeks-old, were used. Hens were randomly assigned to individual cages and separated by an empty cage to avoid stealing feed. Cages were fitted with individual feeding trays. Five hens from each breed were each fed 145 g/day of the CLA-rich diet, and the other five birds received 145 g/day of the control diet. Feed consumption was monitored throughout

the study to ensure that all diets were completely consumed daily. Hens were fed the treatment diet for 16 days, and then returned to the control diet for an additional 16 days.

Eggs were collected from each hen variety and treatment group on days 1, 3, 5, 7, 8, 9, 10, 12, 14 and 16. Duplicate eggs from each hen variety were chosen at random and used to determine CLA accumulation. Similarly, duplicate eggs from each hen variety were collected on days 18, 19, 20, 21, and 23–26 were used to determine CLA depletion from duplicate yolks from each treatment group. All eggs were stored at 4 °C for no more than 36 h before lipid extraction and fatty acid analysis.

Total Lipid Extraction

Total yolk lipids were extracted from duplicate eggs from each collection day and breed using a rapid hexane/isopropanol method [16, 19]. Duplicate 4 g yolk samples were dissolved in 40 mL hexane/isopropanol (1:1, v/v), vortexed for 5 min, and filtered using Whatman #4 filter paper. The solvent was evaporated using a roto-evaporator (Buchi Rotavapor R-210) and extract was weighed.

Fatty Acid Analysis

Fatty acid methyl esters (FAME) were prepared from all extractions using a rapid, micro FAME preparation method [17]. Extraction samples of 0.1 g were weighed (Mettler Toledo Classic AB204-S) in 50 mL centrifuge tubes. A 1 % (wt) heptadecanoic acid methyl ester solution (HME) in hexane was prepared and added at 5 % of the extract weight as an internal standard. One mL of toluene and 4 mL of 0.5 M sodium methoxide in methanol were added to each tube [17]. The centrifuge tubes were heated to 50 °C in a water bath for 10 min and then cooled at ambient temperature for 2 min. Glacial acetic acid, 0.2 mL, was added to each tube to inhibit sodium hydroxide formation. Five mL of distilled water was added, followed by 5 mL of hexane.

The tubes were vortexed for 2 min and then the immiscible layers were allowed to separate. The upper layer was pipetted out and dried over anhydrous sodium sulfate for 15–20 s. Duplicate fatty acid profiles were obtained from each extract by measuring % FAME by GC using an SP 2560 fused silica capillary column (100 m × 0.25 mm i.d. × 2 μm film thickness; Supelco Inc. Bellefonte, PA) equipped with a flame ionization detector (FID model 3800, Varian, Walnut Creek, CA). Samples of 2.0 μL were injected by autosampler. The FID settings were as follows: oven temp = 250 °C; sensitivity = 12, He gas = 30 mL/min, H₂ = 31 mL/min, air = 296 mL/min, initial column temp = 130 °C, final column temp = 225 °C, rate = 0.9 °C/min, and program time = 111 min. Total fatty acid composition, CLA concentration and CLA isomer concentration were calculated by the following equation:

$$[\% \text{ fatty acid}] = [(\text{HME}) \times \text{sample peak area} \times \text{relative response factor}] / \text{HME peak area}$$

Fatty acid percent concentration were converted to mmol of fatty acid/100 g yolk based on the molar mass of each fatty acid and lipid extract as constituting 32 % of total yolk weight. The position of double bonds in CLA-rich soybean oil produced by photoisomerization [13] were previously determined by silver ion HPLC [18].

Statistical Analysis

The following statistical analyses were performed using JMP 11.0.1 software.

CLA concentration in yolk lipids during the steady-state period (days 12–16) were analyzed by 2-way analysis of variance (ANOVA). The LSMEANS, SEM and *P* values of the collection day, breed and day × breed, were determined to verify that CLA concentration stabilized after 12 day of CLA administration.

Quadratic Model

Total yolk CLA accumulation and depletion by breed was first modeled using a 2nd degree polynomial fit, because the data points were in the shape of a parabola. An equivalence test was used ($\alpha = 0.05$) to compare the parameter estimates for the quadratic model from each hen variety.

Piecewise Model

CLA accumulation and depletion were subsequently modeled as separate sub-functions in order to determine if CLA yolk accumulation rates were the same as depletion rates. In addition, accumulation and depletions rates were compared among hen varieties. Non-linear and linear 3-parameter models were separately fit to the accumulation and

depletion segments of the data. The best fitting model was determined by comparing R^2 values, root mean square error (RMSE) values, and residual diagnostics were performed to ensure the final model was adequately representing the data. Predictions from the quadratic model and piecewise model were compared to the observed CLA concentrations. Rates of accumulation and depletion, and total yolk CLA concentration were analyzed by ANOVA in response to breed.

Comparison of *trans,trans* CLA Yolk Accumulation with other CLA Isomers Across Breeds

The isomeric forms of CLA in CLA-rich soybean oil produced by photoisomerization [13] have been previously determined by silver ion HPLC [18]. Individual CLA isomer accumulations were modeled by linear regression, and analyzed by ANOVA as a response of breed and collection day. CLA isomer concentrations were compared using Tukey's honestly significant difference test at α -level of 0.05.

Yolk Fatty Acid Compositional Changes with Respect to Breed and Collection Date

Yolk total fatty acid composition after full CLA accumulation was analyzed by ANOVA in response to breed and blocked by collection date, and compared among varieties using Tukey's honestly significant difference test at α -level of 0.05.

Results

Model Comparisons Describing CLA Accumulation and Depletion

Fatty acid analysis revealed that CLA was not detected in yolks until 6 days after CLA was added to the diets. On day 12 broilers' egg yolks reached a maximum of 0.031 mmol CLA per g of yolk, which was significantly greater ($P < 0.001$) than jungle fowl at 0.027 mmol CLA/g yolk, and Leghorns at 0.023 mmol CLA/g yolk. CLA concentration in yolk lipids from day 12 to 16 did not increase (Table 3). On day 16, CLA supplementation was stopped, and CLA was no longer detected in yolks after day 23. CLA accumulation and depletion were subsequently modeled using both a quadratic model, and a piecewise model in order to determine which best described these rates.

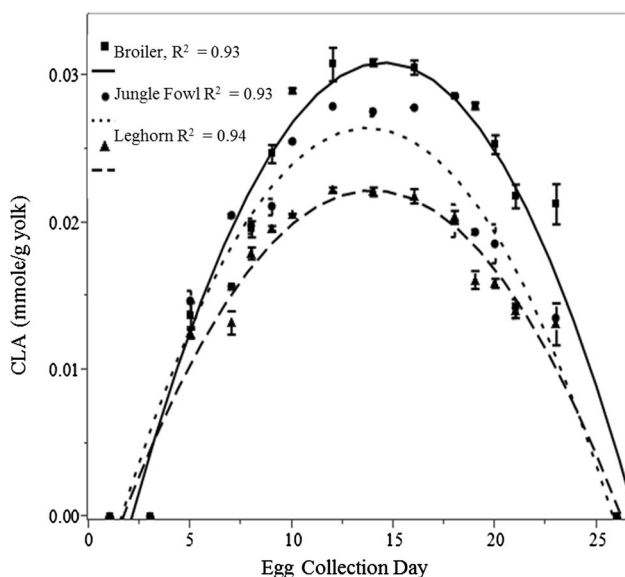
Quadratic Model

Figure 1 shows the total CLA accumulation and depletion in eggs fitted with a quadratic model. Equations 1–3 are

Table 3 Maximum CLA concentration in egg yolks from broilers, jungle fowl and Leghorn hens

	CLA (mmol/g yolk) LSMEAN ± SEM
Broiler	0.031 ± 0.0002a
Jungle fowl	0.028 ± 0.0002b
Leghorn	0.022 ± 0.0002c
	<i>P</i> value
Breed	<0.001
Day (12–16)	0.51
Breed × day	0.91

Two-way ANOVA showed that CLA concentration did not increase after day 12 of treatment administration. Connecting letters indicate values that are not significantly different at 0.05 α -level

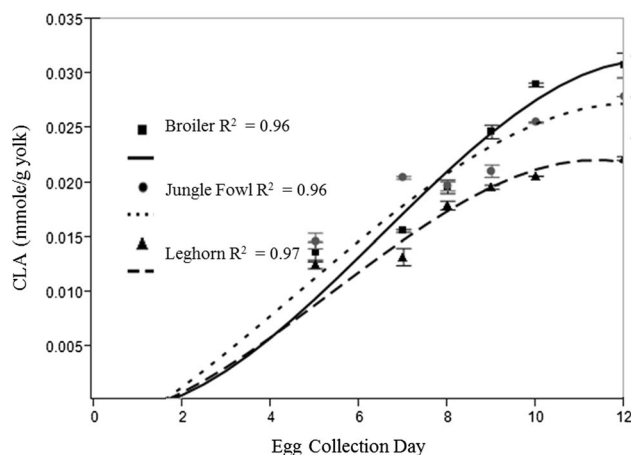
**Fig. 1** Total CLA concentration in yolks from three hen strains during a 16 day administration of CLA-rich soybean oil feed, followed by a 12 day return to a control diet. Data was fitted with a quadratic model

quadratic predictions of the CLA concentration in response to collection day for broilers, jungle fowl, and Leghorns respectively.

$$\begin{aligned} \text{Broiler [CLA] (mmol/g yolk)} \\ = -0.01 + 0.006 \times \text{day} - 0.0002 \times \text{day}^2 \end{aligned} \quad (1)$$

$$\begin{aligned} \text{Jungle fowl [CLA] (mmol/g yolk)} \\ = -0.007 + 0.005 \times \text{day} - 0.0002 \times \text{day}^2 \end{aligned} \quad (2)$$

$$\begin{aligned} \text{Leghorn [CLA] (mmol/g yolk)} \\ = -0.006 + 0.04 \times \text{day} - 0.0001 \times \text{day}^2 \end{aligned} \quad (3)$$

**Fig. 2** Total CLA accumulation in yolks from broilers, jungle fowl, and Leghorns modeled by Gompertz 3-parameter model. Error bars are one standard error from the mean

An equivalence test determined that there were significant differences in rates and total CLA accumulation and depletion among the three breeds ($P < 0.001$). The R^2 values for the broiler, jungle fowl and broiler models were 0.93, 0.93, and 0.94, respectively. The quadratic model accurately described the max CLA accumulation after 12 days of feed administration.

Piecewise Model

The CLA yolk accumulation during days 1 through 12 is shown in Fig. 2. Three different non-linear 3-parameter (3P) models were applied to yolk CLA accumulation data in JMP 11.0 software: cubic model, 3P logistic, and Gompertz 3P model. Using the root mean square error and R^2 values it was determined that CLA yolk accumulation was best modeled by the Gompertz 3P model (Eq. 4). The Gompertz 3P model had the smallest RMSE of all the three-parameter models evaluated at 0.23, while the cubic and logistic models had larger RMSE values of 0.26 and 0.27, respectively. The Gompertz 3P model had an R^2 of 0.96, while the cubic and logistic models' R^2 were lower than 0.95.

The Gompertz 3P model is a time series model where growth is slowest at the start and end of a time period.

$$y(t) = ae^{be^{ct}} \quad (4)$$

where a is the upper asymptote, b is the growth rate, c is the inflection point, and b and c are both negative numbers. Equations 5–7 are the predictive models for CLA accumulation in egg yolks of broilers, jungle fowl, and Leghorns respectively.

$$\text{Broiler[CLA]}(\text{mmol/g yolk}) = 0.036 \times e^{-(0.31 \times e^{-(5.98 \times \text{day})})} \quad (5)$$

$$\begin{aligned} \text{Jungle fowl [CLA] (mmol/g yolk)} \\ = 0.026 \times e^{-(0.57 \times e^{-(4.50 \times \text{day})})} \end{aligned} \quad (6)$$

$$\text{Leghorn [CLA] (mmol/g yolk)} = 0.022 \times e^{-0.48 \times e^{-(4.69 \times \text{day})}} \quad (7)$$

The R^2 values for these three prediction equations were all 0.96. The Gompertz 3P model adequately represents the lag time from day 1 to about day 3 of CLA accumulation, and CLA concentration stabilization after a growth period. These features of the actual data were not apparent in the initial quadratic model, and were not adequately represented by a cubic or logistic three-parameter model (models not shown).

Analysis of means of the three parameters provided in the Gompertz 3P model concluded that: (1) The asymptote estimate for broiler CLA accumulation model was significantly higher than Leghorns and jungle fowl at the 0.05 α -level and (2) there was not a significant difference among CLA accumulation rates at the 0.05 α -level, and (3) The broiler's CLA inflection point was significantly higher than Leghorns and jungle fowl at the 0.05 α -level.

Figure 3 depicts CLA depletion once hens were removed from CLA diets after 16 days of feeding. Depletion was best modeled by a cubic polynomial fit, indicated by the lowest RMSE of 0.16, compared to 0.25 and 0.29 for the Gompertz 3P and logistic 3P models, respectively (models not shown). Equations 8–10 are the cubic descriptive models for CLA depletion in egg yolks from broilers, jungle fowl, and Leghorns respectively.

$$\begin{aligned} \text{Broiler [CLA] depletion (mmol/g yolk)} \\ = 0.45 - 0.06 \times \text{day} + 0.34 \times \text{day}^2 - 0.006 \times \text{day}^3 \end{aligned} \quad (8)$$

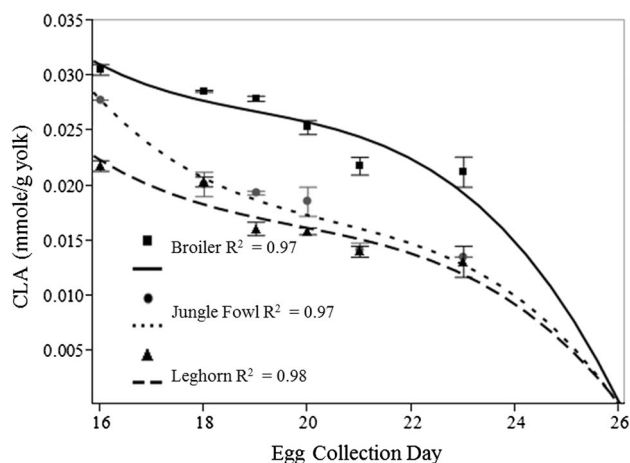


Fig. 3 Total CLA depletion in yolks from broilers, jungle fowl, and Leghorns modeled by a cubic equation. Error bars are one standard error from the mean

$$\begin{aligned} \text{Jungle fowl [CLA] depletion (mmol/g yolk)} \\ = 0.59 - 0.08 \times \text{day} + 0.39 \times \text{day}^2 - 0.006 \times \text{day}^3 \end{aligned} \quad (9)$$

$$\begin{aligned} \text{Leghorn [CLA] depletion (mmol/g yolk)} \\ = 0.38 - 0.05 \times \text{day} + 0.76 \times \text{day}^2 - 0.004 \times \text{day}^3 \end{aligned} \quad (10)$$

The cubic fit also had R^2 values of 0.97 or greater, while the R^2 values for the other two models were less than 0.94. The cubic model seems most appropriate because it best described the steep CLA concentration drop determined in all three breeds after day 23, which corresponds to 7 days after CLA was removed from the diet. While the equivalence test determined differences in all parameter estimates among the three breeds, significant differences in the values were not determined due to so much more variability in CLA concentration among yolk lipids from the same chicken variety during CLA depletion.

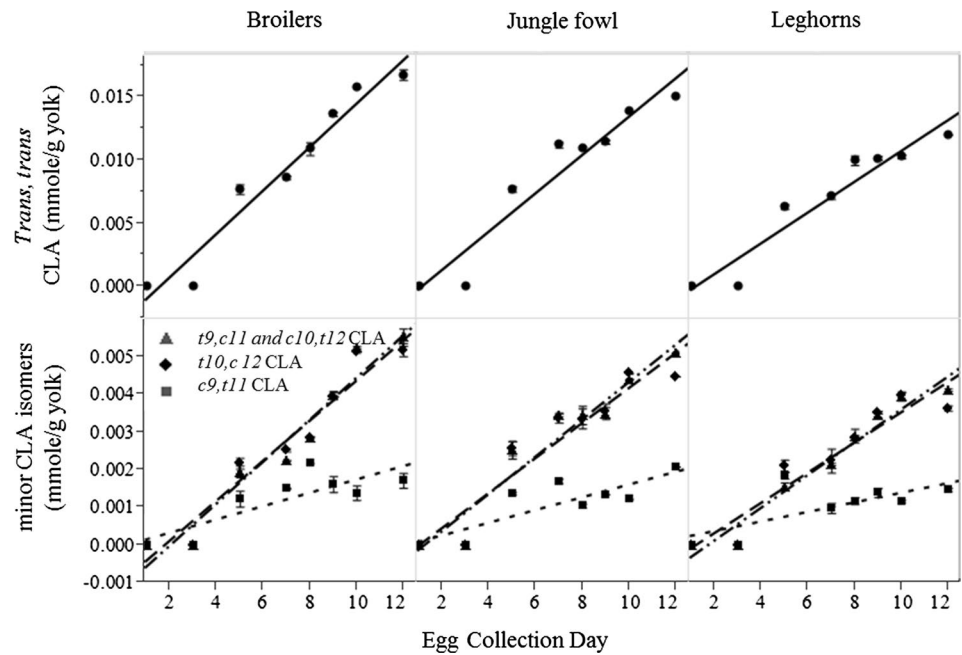
Comparison of trans,trans CLA Yolk Accumulation with other CLA Isomers Across Breeds

Figure 4 shows the accumulation rates of yolk CLA isomers. *Trans, trans* CLA was the dominant isomer throughout CLA accumulation. Broilers contained significantly higher proportions of *t,t* CLA isomers when CLA was first detected on day 5 of the feeding trial at 56.3 % of total CLA ($P < 0.001$). However, *t,t* CLA concentration in broilers decreased to 54.4 % at peak CLA accumulation on day 12, which was not significantly different from *t,t* CLA concentration in Leghorns or jungle fowl, which were also 54 % of total CLA ($P = 0.55$). Jungle fowl and Leghorns' initial *t,t* CLA concentration was 52 % and 50 % of total CLA isomers, respectively. Both breed's eggs experienced a slight increase in relative concentration until peak CLA accumulation on day 12. However, Leghorn's egg yolks *t,t* CLA concentration was very variable throughout the CLA accumulation period. *Trans, trans* CLA isomers in the soybean oil constituted 72 % of total CLA concentration.

Leghorn yolks had the greatest concentration of *c-9, t-11* CLA isomer at 14.7 % of total CLA after 5 days of feeding, but its proportion of total CLA decreased significantly ($P < 0.001$) to 6.5 % after 12 days. There were no significant differences in *c-9, t-11* CLA concentration on day 12 among the three hen strains, ranging from 6–7 % of total CLA ($P = 0.85$). The *c-9, t-11* CLA isomer was only 9 % of total CLA in the CLA-rich soy oil.

The *t-9, c-11* and *c-10, t-12* isomer concentrations were found to be extremely variable throughout the yolk CLA accumulation period, but overall their relative concentration hovered around 16–17 % of total CLA among all three

Fig. 4 Egg yolk CLA isomer concentrations (mmol/g) during CLA accumulation in eggs of three hen strains consuming CLA-rich soybean oil diets. Accumulation rates were modeled by linear regression and comparisons among breed were determined by ANOVA



breeds and all collection days. Broilers had significantly lower concentrations of *t-9,c-11* and *c-10, t-12* isomers ($P = 0.03$). These isomers were 12 % of total CLA in the CLA-rich soybean oil.

The *t-10, c-12* CLA isomers' relative concentration significantly increased during the yolk CLA accumulation period in broiler and Leghorns from initial concentrations of 14 % to reach 18 % of total CLA, while jungle fowl yolks concentration of this isomer did not waiver from 18 % during CLA accumulation. The *t-10, c-12* CLA was only 7 % of total CLA in the CLA-rich soy oil.

During days 12–16 there were no further observed changes in individual CLA isomer concentration. When hens were returned to a control diet on day 16, the concentration of individual CLA isomers during CLA clearance were so sporadic that no meaningful depletion rates for individual isomers could be drawn from the data.

Yolk Fatty Acid Compositional Changes with Respect to Breed and Collection Date

Table 4 shows the total fatty acid composition of RBD soy oil-rich egg yolks and CLA-rich egg yolks from Leghorns, broilers, and jungle fowl after 12 days of feed administration. Soy oil-rich yolks from broilers had significantly lower 18:0 concentrations ($P < 0.05$) than Leghorn yolks, but there were no other discernable differences in control yolk FA composition among hen strains.

Both the RBD and CLA-rich soy oil contain 16 % SFA, but CLA-rich egg yolks had significantly greater saturated fat levels, relative to soy oil-rich yolks. Similarly, MUFA levels in both oils were the same at 26 % of total FA, but

CLA-rich yolks contained significantly lower MUFA levels, relative to control yolks. These fatty acid trends have been similarly reported in every CLA poultry feeding trial [1–6].

In addition, fatty acid concentrations in CLA-rich eggs differed among the three hen varieties (Table 4). Leghorn yolks contained significantly greater amounts of 18:0 relative to both broiler and jungle fowl yolks ($P < 0.001$). Leghorn yolks also had significantly lower 18:2n-6 levels than both jungle fowl and broilers ($P < 0.05$) as well as significantly lower 18:3n-3 concentrations than did broilers ($P < 0.05$). Although total MUFA levels were significantly lower in CLA-rich eggs versus control eggs, their concentrations did not differ among breed ($P = 0.47$), ranging from 30–31 % of total fatty acids. Non-CLA polyunsaturated fatty acid concentrations were significantly lower in Leghorn egg yolks compared to the other breeds ($P < 0.001$). Long chain polyunsaturated fatty acid levels were not different among hen breeds ($P = 0.18$).

Discussion

Model Comparisons Describing CLA Accumulation and Depletion

The quadratic model and piecewise descriptive model both approximate CLA accumulation and depletion very well. However, quadratic models are intrinsically symmetrical, and will indicate that the maximum CLA concentration is reached only at the midpoint day of the feeding trial. In addition, this model assumes that the rates of CLA

Table 4 Yolk total fatty acid composition from Leghorns, broilers, and jungle fowl after 12 days of feeding

Fatty acid (% mole of total)	Leghorn	Broiler	Jungle fowl
(A) RBD soy oil-rich egg yolk fatty acid composition from 3 hen strains			
14:0	0.22 ± 0.02a	0.25 ± 0.03a	0.23 ± 0.02a
16:0	21.6 ± 0.27a	22.4 ± 0.69a	22.0 ± 0.30a
16:1	1.21 ± 0.02a	1.25 ± 0.03a	1.25 ± 0.02a
18:0	11.5 ± 0.27a	10.2 ± 0.30b	10.7 ± 0.36ab
18:1	36.5 ± 0.55a	38.0 ± 0.96a	36.4 ± 0.43a
18:2n-6	25.4 ± 0.81a	23.8 ± 0.62a	24.1 ± 0.29a
18:3n-3	0.78 ± 0.03a	0.85 ± 0.07a	0.80 ± 0.05a
20:4n-6	2.51 ± 0.29a	2.20 ± 0.08a	2.31 ± 0.35a
C22:6n-3	1.60 ± 0.13a	1.51 ± 0.07a	1.6 ± 0.10a
Total saturated fatty acids	33.3 ± 0.36a	32.8 ± 1.31a	32.9 ± 0.51a
Total monounsaturated fatty acids	37.7 ± 0.54a	39.2 ± 0.94a	37.7 ± 0.47a
Total polyunsaturated fatty acids	26.1 ± 0.21a	24.7 ± 0.62a	24.9 ± 0.47a
Total long chain polyunsaturated fatty acids	4.11 ± 0.45a	3.71 ± 0.21a	3.91 ± 0.44a
(B) CLA-rich egg yolk fatty acid composition from 3 hen strains			
14:0	0.25 ± 0.01c	0.40 ± 0.02a	0.32 ± 0.02b
16:0	28.6 ± 0.24a	28.35 ± 0.69a	29.0 ± 0.34a
16:1	0.68 ± 0.05a	0.84 ± 0.06a	0.86 ± 0.06a
18:0	17.4 ± 0.29a	14.0 ± 0.64b	14.7 ± 0.36b
18:1	28.3 ± 0.55a	29.03 ± 0.96a	28.5 ± 0.43a
18:2n-6	18.2 ± 0.2b	20.8 ± 0.62a	20.1 ± 0.29a
18:3n-3	0.45 ± 0.03b	0.76 ± 0.07a	0.58 ± 0.05ab
20:4n-6	2.57 ± 0.29a	1.86 ± 0.08a	2.33 ± 0.35a
22:6n-3	0.82 ± 0.13a	0.51 ± 0.07a	0.6 ± 0.10a
Total saturated fatty acids	48.81 ± 0.36a	44.58 ± 1.31b	46.37 ± 0.51ab
Total monounsaturated fatty acids	29.97 ± 0.58a	31.12 ± 0.84a	30.55 ± 0.47a
Total polyunsaturated fatty acids	20.58 ± 0.27b	23.84 ± 0.52a	22.88 ± 0.47a
Total long chain polyunsaturated fatty acids	3.62 ± 0.43a	2.59 ± 0.11a	3.20 ± 0.47a

Significant differences (α -level 0.05) are shown by connecting letters within the same row

accumulation and depletion are equal, and did not allow a rate comparison. Furthermore, the quadratic model does not account for the empirical observation that CLA levels stabilize after 10–11 days of CLA administration [1–5]. It also does not accurately describe the depletion of CLA from the yolk after CLA was removed from the diets.

Alternatively, the piecewise model provided a more thorough description of accumulation and depletion rates because it incorporates the observed lag phase in CLA accumulation during days 1–4, and adequately describes CLA concentration stabilization at day 11. This model also accurately predicted the maximum yolk CLA concentrations from all hen strains. The piecewise model also describes total CLA depletion in yolks after CLA has been removed from the diet for 7 days.

The fact that both descriptive models were statistically robust shows the importance of observing both the mathematical and biological validity of a model. The piecewise model was not only statistically strong, but also accounted for features of egg laying biology such as 10–12 day cycle

of yolk follicle maturation to full ovulation, which was not accounted for by quadratic modeling. Using a descriptive piecewise model allowed a determination and comparison of both accumulation and depletion rates among different hen varieties, which have not been previously reported. Since all studies that have modified the yolk fatty acid profile by supplementing different fats in the diet have reported a FA composition plateau after 10–12 days, it is likely that this piecewise model can be adapted to most fat-modified feeding trials.

Comparison of *trans,trans* CLA Yolk Accumulation with other CLA Isomers Across Breeds

The *t,t* CLA isomer remained the predominant CLA isomer found in yolks of all three hen strains throughout the accumulation period. Higher concentrations of *t*-10, *c*-12 CLA relative to *c*-9, *t*-11 CLA were observed in the yolk, despite their opposite relative concentrations in the CLA-rich soy oil. This is different from a previous reports that

supplemented equal amounts of *c*-9, *t*-11 and *t*-10, *c*-12 CLA, but observed a greater transfer efficiency of *c*-9, *t*-11 to yolks, [2, 4, 22]. This CLA-rich soy oil seems to be absorbed differently, relative to CLA in free fatty acid form. CLA accumulation resulted in relative proportions of CLA isomers that were similar among the three breeds, with *t,t* CLA at the highest proportion, followed by *t*-10, *c*-12, *t*-9, *c*-11/*c*-10, *t*-12, and *c*-9, *t*-11.

The *t*-10, *c*-12 isomer showed the most substantial increase in its relative proportion of total CLA during yolk accumulation. However, in previous studies, *t*-10, *c*-12 and *c*-9, *t*-11 CLA constituted the majority of the CLA isomers and were at similar concentrations in the diet, but *c*-9, *t*-11 was always preferentially incorporated in the egg yolk at significantly higher concentrations, regardless of breed [1, 3, 5]. Other studies comparing different layer strains' ability to accumulate *c,t t,c* CLA in yolks reported preferential *c*-9, *t*-11 CLA accumulation in white dwarf Leghorns, while the *t*-10, *c*-12 was preferentially incorporated in brown dwarf Leghorns [3, 5].

Yolk Fatty Acid Compositional Changes with Respect to Breed and Collection Date

While the transfer efficiency of the different CLA isomers were similar among hen strain, yolk total fatty acid composition was not consistent among strains. In chickens dietary lipids are transported the liver for further synthesis prior to subsequent tissue and yolk deposition. The stearoyl-CoA desaturase-1 is responsible for converting 16:0 to 16:1 and 18:0 to 18:1 [20]. Previous studies have deemed CLA as an inhibitor of this enzyme, which results in the subsequent increase of SFA in CLA-rich yolks [21, 22]. Broiler hen yolks had the lowest 18:0 levels when fed either the control or CLA-rich diet, relative to other hen strains. In addition, broiler had the greatest 18:2n-6 and 18:3n-3 yolk concentrations relative to the other bird strains. This suggests that CLA stearoyl-CoA desaturase-1 inhibition may not be as prevalent in broilers, relative to Leghorns and jungle fowl.

Overall, broiler CLA-rich egg yolks seemed to exhibit the most desirable fatty acid alterations with the lowest increase in saturated fat concentration, as well as significantly higher MUFA and PUFA levels, and the greatest CLA concentrations in yolks. However, AA and DHA levels were lower in broiler eggs, relative to eggs of the other hen strains. It has been reported that DHA content is lowered in heart and brain tissue of hatched chicks whose mothers received CLA supplementation in free fatty acid form [21]. This study suggests that CLA effects on other tissue FA composition may very well be strain and CLA-source dependent.

It would be interesting to determine a hen strain whose metabolism is optimized for CLA-rich egg and meat

production. CLA-rich soybean oil seems to be absorbed differently than other CLA sources and additional research on muscle and liver deposition is warranted. Furthermore, additional analysis on the specific health benefits of *t,t* CLA is needed before commercial production of this specific isomer is justified.

Descriptive modeling provides a unique insight as to how different CLA isomeric forms are deposited in the yolk, as well as how that deposition changes as maximum yolk CLA concentration is reached. This statistical technique is applicable to most animal-product enrichment studies and may provide additional insight to the researchers and readers in this scientific field.

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