

# Effect of Dietary Conjugated Linoleic Acid on the Quality Characteristics of Chicken Eggs During Refrigerated Storage<sup>1</sup>

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**ABSTRACT** Twenty-four, 79-wk-old White Leghorn hens were assigned randomly to three diets containing 0, 2.5, or 5.0% conjugated linoleic acid (CLA). The diets were fed for 4 wk to determine the effect of dietary CLA on quality characteristics of eggs. Eggs were collected daily and stored at 4 C for 1, 7, 21, or 49 d. At the designated times, the eggs were processed to evaluate water content, fatty acid composition, color, proportions and pH of yolk and albumen. Firmness of yolk after the eggs were hard-cooked was also determined.

The proportions of myristic, palmitic, stearic, CLA (9-*cis*, 11-*trans* CLA and 10-*trans*, 12-*cis* CLA isomers), and unidentified fatty acids in egg yolk lipids were increased as dietary CLA increased, but those of palmitoleic, oleic, linoleic, linolenic, arachidonic, and docosahexaenoic acid were decreased. Duration of refrigeration increased the proportion of egg yolk but decreased the contents of albumen and yolk lipids after 21 d or longer of storage. Egg yolk pH increased as refrigeration time increased, regardless of dietary treatment, but the increase was

greater in the eggs produced by hens fed the CLA diets. Albumen pH increased significantly after 7 d of storage but remained unchanged until 21 d and then decreased by 49 d. Dietary CLA had no effect on the pH of albumen until 49 d of storage. After 49 d storage, egg albumen pH from hens fed CLA diets was lower than that of albumen from hens fed the control diet. Yolk color was not influenced by the dietary CLA and storage, but the egg yolk surface from hens fed CLA diets sometimes had relatively dark color with light spots. Dietary CLA and storage of CLA eggs increased the firmness of hard-cooked egg yolk. The texture of yolks from hard-cooked CLA eggs was rubbery and elastic, and the yolks were more difficult to break using an Instron. It was speculated that the quality changes of CLA eggs were related to the increase of yolk water content, the movement of ions between yolk and albumen through yolk membrane, and the changes of egg yolk pH during storage.

(Key words: dietary conjugated linoleic acid, yolk pH, yolk color, yolk water, firmness)

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## INTRODUCTION

Consumption of conjugated linoleic acid (CLA) has been reported to have several health benefits. Conjugated linoleic acid has been reported to have anticarcinogenic, hypocholesterolemic, and antiatherogenic effects on laboratory animals (Ha *et al.*, 1987; Lee *et al.*, 1994; Ip *et al.*, 1996). Cook *et al.* (1993) reported that CLA enhanced immune responses in rats and prevented the catabolic effects of immune stimulation in rats and chicks. In addition, CLA has been reported to decrease body fat while increasing muscle and bone masses (Dugan *et al.*, 1997; Park *et al.*, 1997; Li and Watkin, 1998). Ha *et al.* (1990) reported that CLA is an effective

antioxidant, more potent than  $\alpha$ -tocopherol and almost as effective as butylated hydroxytoluene (BHT). Pariza and Ha (1990) proposed the antioxidant effect of CLA may be partly responsible for the anticarcinogenic effect of CLA. Dietary CLA isomers are absorbed and incorporated both in adipose and membrane phospholipids (Banni *et al.*, 1996; Kramer *et al.*, 1998), and the accumulation of CLA in animals is dependent upon the amount in the diet and length of intake (Ip *et al.*, 1994; Banni *et al.*, 1995).

Conjugate linoleic acid is found in various food products, including dairy, beef, pork, and poultry products, sea foods, and plant oils (Ha *et al.*, 1989; Chin *et al.*, 1992). The amount of CLA in milk products can be increased by processing or microbial fermentation (Aneja and Murthi, 1990; Shantha *et al.*, 1995). Ip *et al.* (1994) estimated on the basis of a rat model study that a 70-kg human should consume 3.0 g of CLA/d to obtain

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**Abbreviation Key:** BHT = butylated hydroxytoluene; CLA = conjugated linoleic acid.

beneficial effects from CLA. This amount is about threefold higher than the estimated consumption of CLA by an average person in the U.S. (Ip *et al.*, 1994). As a result, there is considerable interest in including CLA in animal feed to improve lean production efficiency and provide value-added healthful animal products for human consumption. Recently, Chamruspollert (1998) reported that feeding diets containing 5% CLA to laying hens resulted in egg yolk fatty acids that contained 11% CLA. These data indicate that CLA-containing eggs could be a dietary source of CLA for humans, assuming that quality characteristics of the eggs were acceptable. However, information on the effects of dietary CLA on the quality of eggs is lacking. The objectives of the study were to determine the effects of dietary CLA concentration on selected quality characteristics of fresh eggs and of eggs refrigerated for different periods of time.

## MATERIALS AND METHODS

### Experimental Animals and Diets

Twenty-four, 79-wk-old White Leghorn hens kept in individual cages were used. Hens were started on the experiment 4 wk after cessation of a forced rest. Each of three dietary treatments was assigned randomly to eight hens. The treatments consisted of diets containing 0, 2.5, or 5.0% CLA. The CLA source<sup>3</sup> contained 65% CLA. The CLA source was in a sealed container (under nitrogen) when received. As the containers were open, 150 mg of ethoxyquin<sup>4</sup> was added/kg of CLA. The CLA source was included at 3.35 and 7.7% of the diet to obtain 2.5 and 5.0% CLA, respectively. The control diet contained 7.7% soybean oil, and the CLA source was substituted for soybean oil to make the other two dietary treatments. Composition of the diet containing soybean oil and the fatty acid composition of the dietary lipids of the experimental diets are presented in Tables 1 and 2, respectively. Feed and water were provided for *ad libitum* consumption throughout the experiment. Body weights were recorded at the start and end of the 28-d trial. Egg production and feed consumption data were recorded daily.

### Sampling and the Storage of Eggs

Results of earlier research (Chamruspollert, 1998) showed that maximum effects of dietary CLA on CLA content of egg yolk lipids occurred at 10 to 11 d after feeding a CLA source began. Thus, beginning on Day 11 of the current experiment, all eggs were labeled according to date of production and hen number, weighed, and stored

at 4 C for 1, 7, 21, or 49 d. For example, eggs produced on Days 11, 12, 13, and 14 of the experiment were stored for 1, 7, 21, or 49 d at 4 C before being analyzed. At the designated times, eggs representing each dietary treatment within a day of production were processed for evaluation of liquid contents and for determination of treatment effects on the firmness of yolks after eggs were hard-cooked. Eggs used for evaluation of yolks and albumen were broken and the weights of yolks, albumens, and shell recorded.

### Total Fat and Fatty Acid Composition of Egg Yolk

Lipids were extracted from samples of each yolk and the diets by the method of Folch *et al.* (1957). Fatty acids of the lipids were methylated by adding 10 mL of anhydrous 3NHCl-methanol to 180 to 200  $\mu$ g of lipid and heating this mixture for 40 min at 60 C (Chin *et al.*, 1992). Concentrations of the methyl esters of fatty acids were determined by gas-liquid chromatography<sup>5</sup> using a Supelcowax-10 fused silica capillary column<sup>6</sup> (30 m  $\times$  0.32 mm inner diameter, 0.25  $\mu$ m film thickness). Oven temperature was programmed at 100 C for 2 min after injection and then was ramped up to 180 C at 20 C/min and to 230 C at 2 C/min. The temperature of 230 C was maintained for 15 min. Temperatures of the injector and flame ionization detector

TABLE 1. Computation of the control, soybean oil-containing diet

Ingredients and analysis	Composition (%)
Corn	38.86
Soybean meal	25.82
Wheat middlings	12.21
Limestone	8.26
Soybean oil <sup>1</sup>	7.70
Meat and bone meal	3.00
Dehydrated alfalfa meal	3.00
Dicalcium phosphate	0.41
Mineral premix <sup>2</sup>	0.30
Vitamin premix <sup>3</sup>	0.30
DL-methionine	0.12
Sodium chloride	0.02
Calculated analysis	
Metabolizable energy, kcal/kg	2,900
Protein	15.0
Total sulfur amino acids	0.62
Methionine	0.35
Lysine	0.75
Calcium	3.65
Nonphosphate P	0.35
Sodium	0.18
Ether extract	8.94

<sup>1</sup>Source of conjugated linoleic acid (CLA) was substituted for 50 or 100% of the soybean oil to obtain diets calculated to contain 2.5 or 5.0% CLA, respectively.

<sup>2</sup>Supplied the following per kilogram of diet: manganese, 80 mg; zinc, 90 mg; iron, 60 mg; copper, 12 mg; selenium, 0.147 mg; sodium chloride, 2.247 g.

<sup>3</sup>Supplied the following per kilogram of diet: vitamin A (retinyl acetate), 8,060 IU; cholecalciferol, 1,580 IU; vitamin E (dl- $\alpha$ -tocopheryl acetate), 15 IU; vitamin B<sub>12</sub>, 16  $\mu$ g; vitamin K (menadine dimethylpyrimidinol bisulfate), 4 mg; riboflavin, 7.8 mg; pantothenic acid, 12.8 mg; niacin, 75 mg; choline, 509 mg; folic acid, 1.6 mg; biotin, 0.27 mg.

<sup>6</sup>Supelco, Belfonte, PA 16823-0048.

<sup>3</sup>Pharmanutrients, Inc., Lake Bluff, IL 60044.

<sup>4</sup>Sigma Chemical Co., St. Louis, MO 63178-9916.

<sup>5</sup>HP 6890, Hewlett Packard Co., Wilmington, DE 16808-1610.

TABLE 2. Composition of lipids in diets containing 0, 2.5, or 5.0% conjugated linoleic acid (CLA)

Fatty acids	Dietary CLA concentration		
	0%	2.5%	5.0%
	————— (% of diet) —————		
Myristic	ND <sup>1</sup>	0.01	0.02
Palmitic	1.04	0.73	0.57
Palmitoleic	0.02	0.02	0.02
Stearic	0.30	0.22	0.18
Oleic	1.83	1.86	1.85
Linoleic	4.15	2.22	1.05
Linolenic	0.56	0.25	0.05
CLA	ND	2.77	4.26
	————— (% of total methyl esters) —————		
Myristic	ND	0.12	0.25
Palmitic	13.17	9.04	7.12
Palmitoleic	0.25	0.25	0.25
Stearic	3.80	2.72	2.25
Oleic	23.16	23.02	23.18
Linoleic	52.53	27.48	13.12
Linolenic	7.09	3.09	0.62
CLA	ND	34.28	53.26

<sup>1</sup>Not detected.

were 220 and 250 C, respectively. Results of fatty acid analyses were compared qualitatively with Nu Chek<sup>7</sup> fatty acid methyl ester standard GLC-63, GLC-91, UC-59-M, and UC-72-A.

### Color and pH Measurements

Color of egg yolk was determined using a 10-point Improved Roche Color Fan.<sup>8</sup> Egg yolk was separated from the white, and the closest matching color with the color fan was given as a yolk color. When the color of yolk was a darker yellow color than the color in the scale, an 11-point score was given. The pH of the egg yolk or white was measured after diluting the sample with 9 vol of deionized distilled water.

### Firmness

Eggs to be used to determine the influence of dietary CLA and duration of refrigerated storage on firmness of hard-cooked yolks were taken from storage, allowed to attain room temperature, and cooked in water for 15 min after the water came to a boil. Cooked eggs were cooled to room temperature and the yolks separated from the shell and albumen. The resistance of each yolk to compression was determined by using an Instron with Series 12 Cyclic Test Software.<sup>9</sup> The Instron was equipped with a 35 mm anvil, and the cross-head speed was 7 cm/min. Resistance to compression was measured as kilograms of peak load encountered while compressing the yolks to 80% of their original height.

### Statistical Analysis

Data were analyzed according to a split-plot design, with dietary CLA concentration as the whole plot and duration of egg storage as the sub-plots, to determine main effects of dietary CLA and duration of storage, and their interactions. Tukey's test was used to compare treatment and main effect means, as appropriate. When dietary CLA by duration of refrigeration interactions were observed, comparisons among treatments were made. Otherwise, comparisons were made among main means.

### RESULTS AND DISCUSSION

Hens fed the diet containing 5% CLA consumed less feed ( $P < 0.01$ ) than hens fed 0 or 2.5% CLA (Table 3). Rate of egg production and body weight gain also were adversely affected by feeding 5% CLA. However, no effects of dietary CLA concentration on feed efficiency or egg weight were detected. No mortality of hens occurred during the trial.

Because there were no interactions between dietary CLA concentration and duration of refrigeration, only main effect means of the fatty acid concentrations of yolk lipids are presented. Except for eicosanoic acid, the proportions of all fatty acids in egg yolk lipids were significantly ( $P < 0.01$ ) influenced by the dietary CLA (Table 4). The proportions of myristic, palmitic, stearic, CLA (9-*cis*, 11-*trans* CLA and 10-*trans*, 12-*cis* CLA), and unidentified fatty acids in egg yolk lipids were increased by dietary CLA, but those of palmitoleic, oleic, linoleic, linolenic, arachidonic, and docosahexaenoic acid were decreased. These changes in fatty acid composition of yolk lipids are similar to those reported by Chamruspollert (1998), although the total CLA concentration observed in the current research when 5% CLA was fed (8.5 to 8.6%) was less than the 11.2% reported by Chamruspollert (1998). The decrease in the concentrations of linoleic and linolenic acids in yolk lipids of hens fed CLA probably reflects the relatively low concentration of these fatty acids in the CLA source as compared with soybean oil. Decreases in arachidonic acid and docosahexaenoic acid in yolk lipids from hens fed CLA also could be related to the low concentration of dietary linoleic and linolenic acids, which serve as precursors to the formation of arachidonic and docosahexaenoic acids. Another possibility is that CLA may compete with linoleic or linolenic acid for  $\Delta 6$ -desaturase, the rate-limiting step for the conversion of these fatty acids into arachidonic acids or docosahexaenoic acid in liver microsomes (Belury and Kempa-Steczko, 1997). Feeding CLA increased the concentration of stearic acid in yolk lipids. Lee *et al.* (1995) found that rabbits fed a CLA diet had a higher ratio of saturated fatty acids, especially palmitic and stearic, to monosaturated fatty acids, palmitoleic and oleic, in plasma, liver and aorta tissues. They suggested that CLA inhibited the  $\Delta$ -desaturase

<sup>7</sup>Nu Chek Prep., Inc., Elysian, MN 56028.

<sup>8</sup>Hoffmann LaRoche, Nutley, NJ 07110.

<sup>9</sup>Instron Corp., Canton, MA 02021.

**TABLE 3. Influence of dietary conjugated linoleic acid (CLA) on performance and change in body weight of laying hens**

Percentage dietary CLA	Feed consumption	Rate of egg production	Feed efficiency	Body weight gain	Egg weight
(%)	(g/hen/d)	(%)	(kg:kg)	(g/hen)	(g/egg)
0	103.7 <sup>ab</sup>	77.0 <sup>ab</sup>	2.06	79 <sup>a</sup>	65.5
2.5	111.4 <sup>a</sup>	82.6 <sup>a</sup>	2.07	86 <sup>a</sup>	64.9
5.0	92.9 <sup>b</sup>	72.8 <sup>b</sup>	1.96	-5 <sup>b</sup>	65.1
SEM	3.7	2.1	0.16	20	1.6
Probabilities					
Diet effect <sup>1</sup>	0.008	0.012	0.058	0.015	0.31

<sup>a,b</sup>Column means with no common superscript differ significantly ( $P < 0.05$ ).

<sup>1</sup>Cumulative data for a 28-d experimental period, eight hens per dietary treatment.

**TABLE 4. Influence of dietary conjugated linoleic acid (CLA) and days of refrigeration on fatty acid composition of egg yolk lipids**

Fatty acids	Duration of refrigeration						SEM	Probabilities of diet effect
	1 d			49 d				
	0% CLA	2.5% CLA	5.0% CLA	0% CLA	2.5% CLA	5.0% CLA		
(%)	(% of total methyl esters)							
Myristic	0.16	0.42	0.42	0.22	0.41	0.41	0.04	0.001
Palmitic	22.82	27.34	26.70	23.00	27.50	27.47	0.31	0.001
Palmitoleic	1.86	0.85	1.08	1.71	0.81	1.03	0.06	0.001
Stearic	11.06	16.00	16.12	11.80	15.98	16.77	0.38	0.001
Oleic	32.36	22.43	25.19	32.21	21.95	24.07	0.53	0.001
Linoleic	24.68	22.09	15.18	23.43	22.37	14.72	0.62	0.001
Linolenic	1.45	1.07	0.23	1.37	1.07	0.32	0.06	0.001
9- <i>cis</i> , 11- <i>trans</i> CLA	ND <sup>1</sup>	2.50	4.30	ND	2.59	4.46	0.16	0.001
10- <i>trans</i> , 12- <i>cis</i> CLA	ND	2.31	4.32	ND	2.20	4.04	0.14	0.001
Eicosaenoic	ND	0.08	0.13	ND	0.02	0.09	0.05	0.085
Arachidonic	3.64	2.95	2.64	3.74	2.72	2.47	0.15	0.001
Docosahexaenoic	1.96	0.85	0.24	2.06	0.82	0.34	0.10	0.001
Unidentified	0.01	1.11	3.45	0.46	1.56	3.81	0.22	0.001

<sup>1</sup>ND = not detected.

**TABLE 5. Influence of dietary conjugated linoleic acid (CLA) and days of refrigeration on percentage of yolks, albumens, and shells of eggs and lipid concentration of yolks<sup>1</sup>**

Duration of refrigeration <sup>2</sup>	Yolk				Albumen				Shell				Yolk lipids			
	0% CLA	2.5% CLA	5.0% CLA	$\bar{x}$	0% CLA	2.5% CLA	5.0% CLA	$\bar{x}$	0% CLA	2.5% CLA	5.0% CLA	$\bar{x}$	0% CLA	2.5% CLA	5.0% CLA	$\bar{x}$
(d)	(% of egg weight)															
1	28.1 <sup>de2</sup>	27.7 <sup>def</sup>	26.8 <sup>ef</sup>	27.5	61.6 <sup>ab</sup>	61.9 <sup>ab</sup>	62.5 <sup>ab</sup>	62.0	10.3	10.4	10.7	10.5	35.0	35.9	35.2	35.4
7	27.8 <sup>def</sup>	29.4 <sup>cd</sup>	26.1 <sup>f</sup>	27.8	61.7 <sup>ab</sup>	60.1 <sup>cd</sup>	63.3 <sup>a</sup>	61.7	10.5	10.5	10.6	10.5	34.8	34.9	35.3	35.0
21	28.4 <sup>cde</sup>	29.0 <sup>cd</sup>	28.8 <sup>cd</sup>	28.7	61.3 <sup>bcd</sup>	60.5 <sup>cd</sup>	60.5 <sup>cd</sup>	60.8	10.3	10.5	10.7	10.5	35.1	33.5	33.6	34.1
49	30.0 <sup>bc</sup>	32.0 <sup>a</sup>	31.2 <sup>ab</sup>	31.1	59.6 <sup>d</sup>	57.7 <sup>e</sup>	58.5 <sup>de</sup>	58.6	10.4	10.3	10.3	10.3	33.9	31.3	32.1	32.4
$\bar{x}$	28.6	29.5	28.2		61.0	60.0	61.2		10.4	10.4	10.6		34.7	33.9	34.0	
SEM	0.35				0.37				0.15				0.61			
Probabilities																
Diet (D)	0.001				0.001				0.17				0.50			
Days (DA)	0.001				0.001				0.42				0.001			
D × DA	0.001				0.001				0.71				0.36			

<sup>a-f</sup>Means with no common superscript differ significantly ( $P < 0.05$ ).

<sup>1</sup>Means of 7 to 16 eggs.

<sup>2</sup>Refrigerated at 4 C.

TABLE 6. Influence of dietary conjugated linoleic acid (CLA) and duration of refrigeration on water content and pH of egg yolk and pH of egg albumen<sup>1</sup>

Duration of refrigeration <sup>2</sup>	Yolk water				Yolk pH				Albumen pH				Yolk color <sup>3</sup>			
	0% CLA	2.5% CLA	5.0% CLA	$\bar{x}$	0% CLA	2.5% CLA	5.0% CLA	$\bar{x}$	0% CLA	2.5% CLA	5.0% CLA	$\bar{x}$	0% CLA	2.5% CLA	5.0% CLA	$\bar{x}$
(d)	(%)															
1	40.52 <sup>2</sup>	41.36	43.59	41.86	6.20 <sup>d</sup>	6.20 <sup>d</sup>	6.21 <sup>d</sup>	6.20	9.05	9.01	9.01	9.02	9.50	10.00	10.00	9.83
7	43.71	43.99	45.58	44.48	6.26 <sup>d</sup>	6.30 <sup>d</sup>	6.36 <sup>d</sup>	6.31	9.36	9.36	9.35	9.36	10.00	9.75	9.50	9.75
21	45.51	47.50	48.40	47.25	6.28 <sup>d</sup>	6.48 <sup>d</sup>	6.96 <sup>c</sup>	6.57	9.24	9.32	9.35	9.30	9.56	9.40	10.00	9.65
49	46.15	50.82	52.36	49.67	6.33 <sup>d</sup>	7.33 <sup>b</sup>	8.19 <sup>a</sup>	7.28	8.99	8.69	8.60	8.76	9.06	9.56	10.43	9.68
$\bar{x}$	43.68	45.48	46.94		6.27	6.58	6.93		9.16	9.10	9.08		9.53	9.68	9.98	
SEM		0.81				0.07				0.08				0.30		
	Probabilities															
Diet (D)	0.001				0.001				0.13				0.30			
Days (DA)	0.001				0.001				0.001				0.49			
D × DA	0.69				0.001				0.46				0.18			

<sup>a-d</sup>Means within a variable with no common superscript differ significantly ( $P < 0.05$ ).

<sup>1</sup>Means of 7 to 16 eggs.

<sup>2</sup>Refrigerated at 4 C.

<sup>3</sup>Scored on the basis of the Improved Roche Color Fan, Hoffmann LaRoche, Nutley, NJ 07110.

enzyme system in the liver, thereby increasing saturated fatty acid concentrations.

Percentage of yolks was increased as dietary CLA ( $P = 0.001$ ) or storage time increased ( $P < 0.001$ ) (Table 5). The greatest changes during storage occurred with eggs from hens fed 2.5 or 5.0% CLA, resulting in a dietary CLA by storage time interaction ( $P = 0.001$ ). Percentage of albumen decreased ( $P < 0.001$ ) with time in refrigerated storage, especially between 7 and 49 d. Albumen percentage also was significantly ( $P < 0.001$ ) affected by dietary CLA, resulting in a CLA by duration of refrigeration interaction. Proportion of eggshell was not influenced by CLA or time of refrigeration. Percentage of lipids in yolks decreased ( $P < 0.001$ ) with time of refrigeration, with greatest decreases occurring between 7 and 49 d. The changes of lipid concentration in yolks during storage were not caused by dietary CLA but by the interaction with storage time. Increases in the proportion of yolk in eggs during storage were accompanied by decreases in the proportion of albumen, suggesting that some component of albumen may have moved into the yolk, especially in eggs produced by hens fed CLA.

Concentration of water in yolks varied widely (40.5 to 52.4%) and were lower than the reported values of 47 to 50% (Marion *et al.*, 1964; Kline *et al.*, 1965; Ahn *et al.*, 1997) except for the eggs from hens fed CLA diets and stored 3 wk or longer (Table 6). Water concentration in yolk increased as dietary CLA ( $P < 0.05$ ) or duration of storage increased ( $P < 0.05$ ), and increase in yolk water with storage were consistent and correlated well with changes in proportion of yolk in the eggs (Table 5). However, no interaction between dietary CLA and storage time on water concentration in yolk was found. Rose *et al.* (1966) reported that yolk water can vary by the age of the laying hen. During the storage of shell eggs, water migrates from the albumen to the yolk, and,

as a consequence, the yolk water content increases (Stadelman and Cotterill, 1986).

Egg yolk pH increased during refrigerated storage in all dietary treatments, but the increase was greatest in the eggs from hens fed CLA diets, resulting in an interaction ( $P < 0.001$ ). After 49 d of storage, the pH of egg yolk from hens fed 5.0% CLA was about 2 pH units higher than that of hens fed the control diet (Table 6). The yolk pH in freshly laid eggs is generally about 6.0, but during storage of eggs the pH gradually increases to between 6.4 and 6.9 in about 50 d (Stadelman and Cotterill, 1986). The pH of yolks from hens fed the control diet were lower than the literature values but the pH of yolks from hens fed the CLA diets were much higher than the literature values after 49 d of refrigera-

TABLE 7. Influence of dietary conjugated linoleic acid (CLA) and days of refrigeration on firmness of egg yolks from hard-cooked eggs<sup>1</sup>

Duration of refrigeration <sup>2</sup>	Yolk firmness <sup>3</sup>			
	0% CLA	2.5% CLA	5.0% CLA	$\bar{x}$
(d)	(kg)			
1	4.40 <sup>e</sup>	5.12 <sup>de</sup>	6.66 <sup>d</sup>	5.39
7	4.14 <sup>e</sup>	7.12 <sup>d</sup>	9.69 <sup>c</sup>	6.98
21	4.31 <sup>e</sup>	10.25 <sup>bc</sup>	11.67 <sup>bc</sup>	8.74
49	4.26 <sup>e</sup>	12.01 <sup>b</sup>	14.74 <sup>a</sup>	10.34
$\bar{x}$	4.28	8.62	10.69	
SEM	0.47			
	Probabilities			
Diet (D)	0.001			
Days (DA)	0.001			
D × DA	0.001			

<sup>a-e</sup>Means with no common superscript differ significantly ( $P < 0.05$ ).

<sup>1</sup>Means of 9 to 36 eggs.

<sup>2</sup>Refrigerated at 4 C.

<sup>3</sup>Firmness determined by Instron Model 4502, Instron Corp., Canton, MA 02021.

tion. These values indicate that ion movement between the yolks and albumen through the yolk membrane in CLA eggs was greater than that in the control eggs. It is speculated that the incorporation of CLA, most of which was in the *trans* form, could have increased the permeability of yolk membrane because of the *cis-trans* arrangements of CLA, which would have increased membrane fluidity and sizes of space between the fatty acid chains of phospholipids that constitute the membrane bilayer.

Albumen pH increased significantly after 7 d of storage but remained unchanged until 21 d and then was lower at 49 d. Dietary CLA had no effect on the pH of albumen. At 49 d of storage, egg albumen pH from hens fed CLA diets was lower than that of albumen from hens fed the control diet (Table 6). The pH of albumen in 1-d-old eggs in this study was rather high considering that the pH of freshly laid eggs is usually between 7.6 to 8.5 (Heath, 1975, 1977). However, the pH of egg albumen has been shown to increase to 9.18 after 3 d of storage at 3 C (Stadelman and Cotterill, 1986). This increase in pH indicates that albumen pH can be increased rapidly under refrigerated conditions, although the rate of pH changes in egg albumen is temperature-dependent. There was no influence of time in refrigerated storage on yolk color (Table 6). The effect of dietary CLA was inconsistent and not significant ( $P > 0.05$ ).

Data presented in Table 7 show that as dietary CLA concentration and time of refrigeration increased, the firmness of yolks from hard-cooked eggs increased. An interaction between dietary CLA and storage also was observed, whereby yolk firmness increased more with longer refrigeration time as dietary CLA increased. The textures of hard-cooked CLA egg yolks were rubbery and elastic, and the hard-cooked yolks were relatively difficult to break using an Instron. We assume that the textural changes of CLA eggs could be related to the increase of yolk water content, the movement of ions between yolk and albumen through yolk membrane, or changes of egg yolk pH during storage. However, it is impossible to pinpoint the causes of such changes in CLA eggs at this time.

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