Increasing Content of Healthy Fatty Acids in Egg Yolk of Laying Hens by Cheese Byproduct

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ABSTRACT : This study investigated the effects of dietary supplementation of cheese byproduct on performance, egg quality and fatty acid profile of egg yolk lipids from laying hens. One hundred five 30-wk-old White leghorn laying hens were randomly distributed into five groups of twenty one hens each and maintained in individual laying cages for 4 weeks. The hens were assigned to five treatments that consisted of corn-soybean meal based diets containing 0, 1, 3, 5 or 10% of cheese byproduct. Feed intake and rate of egg production of hens were not significantly different across the treatments during the whole experiment ($p>0.05$). Similarly, egg yolk cholesterol level, egg weight, Haugh's unit, eggshell thickness, color, and strength were not significantly different across the treatments ($p>0.05$). The amount of C16:0 in egg yolk was not significantly different across the treatments, but that of C18:0 decreased with increased cheese byproduct ($p<0.01$). Monounsaturated fatty acid (C16:1 and C18:1) content in egg yolk was similar across the treatments. Total CLA and cis-9, trans-11 CLA content increased linearly with increased cheese byproduct ($p<0.001$), while trans-10, cis-12 CLA amount was not significantly different across the treatments ($p>0.05$). Total saturated fatty acid (SFA) in the egg yolk was decreased as the level of cheese byproduct including CLA increased ($p<0.01$). However, the amount of unsaturated fatty acids (UFA) such as monounsaturated fatty acids (MUFA), n-3 polyunsaturated fatty acids (PUFA), n-6 PUFA, and total PUFAs in the egg yolk were not significantly different across the treatments ($p>0.05$). Therefore, the present results showed that cheese byproduct beneficially improved the fatty acid composition of concern to human health in the egg yolk without adverse effects on egg quality. ***(Asian-Aust. J. Anim. Sci. 2006. Vol 19, No. 3 : 444-449)***

Key Words : CLA, Fatty Acid, Cheese Byproduct, Egg, Hen

INTRODUCTION

Recently, there have been a lot of interests in improving the quality of food products of animal origin with conjugated linoleic acids (CLA) due to their beneficial effects on human health. Various researchers have demonstrated beneficial effects of CLA on human health such as anticarcinogenic (Ip et al., 1999; Scimeca, 1999), hypolipidemic (Yeung et al., 2000), antiatherosclerotic (Lee et al., 1994; Nicolosi et al., 1997), antiadipogenic (Park et al., 1999; Choi et al., 2004), and immune-enhancing (Cook et al., 1993) effects. Conjugated linoleic acid is abundant in food products from ruminant animals (Ha et al., 1989; Chin et al., 1992; Shantha et al., 1992), because it is formed as an intermediate during biohydrogenation of C18:2 n-6 to C18:0 by rumen bacteria such as *Butyrivibrio fibrisolvens* (Kepler et al., 1966). There have been considerable efforts to increase natural CLA contents in food products by the manipulation of feeding regimen (Choi et al., 2002; Kim et al., 2003) and *M. elsdeniae* (Kim et al., 2005). For this reason, cheese or cheese byproduct, which is a dairy product, was thought to be used as a good dietary CLA source. Although CLA level in the cheese varies by the processing condition, the CLA is ranged between 1.2-2.6 mg/g sample (Jiang et al., 1997; Lin et al., 1995; Ma et al., 1999). On the other hand, it was reported that trans-10, cis-9 CLA level is very low in the various cheese types (Zlatanos et al., 2002), However, there has been considerable efforts to enrich CLA in laying hen's feed to improve its content in eggs for human consumption (Ahn et al., 1999; Chamruspollert and Sell, 1999; Hwangbo, 2005).

Therefore, the objectives of this experiment were to test the possibility of healthy fatty acids such as CLA and UFAs enforced egg production without any adverse effects using cheese byproducts as a CLA source.

MATERIALS AND METHODS

Birds, diets and protocol

One hundred five 30-wk-old White leghorn laying hens were randomly distributed into five groups of twenty one hens for each group maintained in individual laying cages during 4 weeks. For experimental diets, corn-soybean meal based diets containing 0, 1, 3, 5 and 10% of cheese byproduct were used. The ingredient and chemical composition of the experimental diets are shown in Table 1.

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Table 1. Formulation and chemical composition of the experimental diets

	Cheese byproduct				
	0%	1%	3%	5%	10%
Ingredient (%)					
Corn	71.00	71.00	69.00	68.00	65.00
Soybean meal	16.50	15.50	15.50	14.50	12.50
Gluten meal	3.00	3.00	3.00	3.00	3.00
Salt	0.30	0.30	0.30	0.30	0.30
Limestone	7.30	7.30	7.30	7.30	7.30
Vitamin-Premix[1]	0.50	0.50	0.50	0.50	0.50
L-Lysine	0.20	0.20	0.20	0.20	0.20
Methionine	0.20	0.20	0.20	0.20	0.20
Bicalcium phosphate	1.00	1.00	1.00	1.00	1.00
Cheese byproduct[2]	-	1.00	3.00	5.00	10.00
Chemical composition (%)					
Dry matter	87.9	87.9	87.1	86.7	85.1
Ash	7.13	7.01	6.83	6.93	6.87
Crude protein	12.74	13.26	13.42	12.85	13.25
Ether extract	1.39	1.59	1.94	2.44	3.31
Crude fiber	1.49	1.45	1.72	1.47	1.40
Calcium	3.98	3.28	3.68	4.12	4.14
Phosphate	0.63	0.45	0.40	0.47	0.51
ME (Cal/g)	3,530	3,496	3,516	3,603	3,490

[1] Provided per kg diet: Vitamin A, 5,500 IU; Vitamin D_3, 1,100 IU; Vitamin E, 11 IU; Vitamin B_{12}, 0.0066 mg; Riboflavin, 4.4 mg; Niacin, 44 mg; Pantothenic acid, 11 mg (Ca-pantothenate, 11.96 mg); Choline, 190.96 mg (Choline chloride 220 mg); Menadione, 1.1 mg (Menadione sodium bisulfite complex, 3.33 mg); Folic acid, 0.55 mg; Pyridoxine, 2.2 mg (Pyridoxine hydrochloride, 2.67 mg); Biotin, 0.11 mg; Thiamin, 2.2 mg (Thiamine mononitrate, 2.40 mg); Ethoxyquin, 125 mg; Cu, 30; Zn, 60; Mn, 90; Co, 0.25; I, 1.2; Se, 0.3.

[2] Cheese byproduct contains: Crude protein, 24 g/100 g; Ether extract, 26 g/100 g; Na, 373 mg/100 g; Ca, 517 mg/100 g; P, 371 mg/100 g; Fe, 0.2 mg/100 g; K, 67.0 mg/100 g.

Table 2. Fatty acid composition in experimental diets (%)

Fatty acid	Cheese byproduct				
	0%	1%	3%	5%	10%
C10:0	ND	0.27	0.92	1.15	1.41
C12:0	0.01	0.42	1.43	1.77	2.30
C14:0	0.11	1.25	4.03	4.86	7.32
C16:0	15.06	16.79	21.00	22.22	25.69
C18:0	2.45	3.74	6.50	7.14	9.50
C18:1 n-9	28.40	28.41	27.84	28.27	27.85
C18:2 n-6	51.00	45.82	33.47	29.93	27.37
C18:3 n-3	1.87	1.69	1.22	1.04	1.03
CLA c9 t11	ND	ND	0.20	0.20	0.28
CLA t0c12	ND	ND	ND	ND	ND
Total CLA	ND	0.54	0.66	0.73	0.80

ND; Not detected.

The cheese byproduct (Haitai Dairy Co., Ltd. Suwon, Korea) as a CLA source contained 1.41 g CLA/kg. The cheese byproduct was obtained from cheese processing, and then it is cheese itself. Cheese byproduct contained 11% of C14:0, 31% of C16:0, 13% of C18:0, 26% of C18:1, 3% of C18:2, 0.2% of C18:3 and 0.8% of CLA. The fatty acid composition of experimental diets including different level of cheese by-product is given in Table 2. The proportions of SFA such as C10:0, C12:0, C14:0, C16:0 and C18:0 were relatively higher in the treatments including cheese byproduct compared with control. As expected, the proportion of CLA was increased with increased amount of cheese byproduct amount, but no CLA was detected from in the control diet. The proportion of C18:1 n-9 was similar across the diets including different levels of cheese byproduct. Unlike to C18:1 n-9, C18 polyunsaturated fatty acids such as C18:2 n-6 and C18:3 n-3 proportions were relatively lower in the diets including cheese byproduct compared with the control.

Feed and water were available *ad libitum*. The photoperiod was set at 17L:7D during the experiment. Eggs were colleted and counted daily to obtain egg production, and feed consumption for each replicate was recorded daily for the entire study. Collected eggs were broken open, and examined the egg quality twice per week, and then frozen at -20°C for further analyses.

Analysis

Egg weight, Haugh's unit (HU), and egg yolk color were measured with QCM$^+$ (Technical Services and Supplies, York, England), and eggshell thickness and strength were measured with FHK (Fujihara Co. LTD, Saitama, Japan). Egg yolk cholesterol was determined by enzymatic assays using kits (Wako Pure Chemical Industries, Ltd., Japan).

Lipid from egg yolks was extracted with hexane/isopropanol (3:2 v/v). Fatty acids were converted into methyl esters with modification to the method

Table 3. Effect of dietary cheese byproduct on performance and egg quality of hens

	Cheese byproduct					SEM	Significance
	0%	1%	3%	5%	10%		
Feed intake (g/d)	104.8	105.6	105.5	106.9	110.8	7.03	NS
Rate of egg production (%)	79.0	80.5	78.3	78.0	80.1	12.65	NS
Cholesterol (g/100 g egg yolk lipid)	4.24	4.45	4.50	4.50	4.91	0.85	NS
Egg weight (g/egg)	60.25	57.52	58.73	57.21	61.45	7.71	NS
Haugh's unit: HU	82.43	84.67	75.50	82.17	77.87	8.38	NS
Eggshell Thickness (mm)	0.36	0.34	0.34	0.34	0.32	0.04	NS
Egg yolk color	8.00	8.28	8.17	8.00	8.33	1.02	NS
Eggshell strength (kg/m^2)	4.74	4.32	4.14	4.15	4.08	1.19	NS

NS; Not significant.

described in our previous report (Kim and Liu, 1999). Briefly, 0.5 ml of toluene and 2 ml of 5% KOH-MeOH added to the lipid, and the sample vortex-mixed and heated at 70°C for 8 min. The sample was cooled in cold water, 2 ml of 14% BF3-MeOH was added to the sample, and the sample vortex-mixed and heated at 70°C for 8 min. The sample was cooled in cold water, 3 ml of 5% NaCl (for saturation) added to the sample and vortex-mixed. Five mL of distilled water and 0.5 ml of hexane added to extract the FAME. The mixture was shaken for 5 min and centrifuged at 3,000 rpm for 5 min, and then the upper phase was collected, dried with sodium sulfate.

Samples were analyzed for CLA isomers and total fatty acid profile using an HP5890 gas chromatograph with a flame ionization detector (Hewlett Packard 5890 Series II). Fatty acid methyl esters were separated using a Supelcowax-10 fused silica capillary column (60 m×0.32 mm i.d., 0.25 μm film thickness; Supelco, Inc., Bellefonte, PA, USA) with a 1.2 ml/min of helium flow. Oven temperature was increased from 230 to 240°C at the rate of 2°C/min. Temperatures of the injector and detector were 240 and 250°C, respectively. One μl of sample was injected into the column in the split mode (50:1). The peak of each CLA isomer (cis-9 trans-11, trans-10 cis-12, cis cis, and trans trans isomer) and other fatty acids were identified and quantified by comparison with the retention time and peak area of each fatty acid standard (Sigma) respectively. Fatty acid content was expressed as % of total fatty acids. Heptadecanoic acid (C17:0) was used as an internal reference before the extraction to determine the recovery of the fatty acids in the samples. The recovery of methylated fatty acids that was analyzed in a comparison to the internal standard was higher than 80%.

Statistical analysis

Statistical differences were determined by an analysis of variance, with mean separations performed by the Duncan multiple range test range test using the general linear model procedure of the SAS statistical software (SAS, 1996).

RESULTS AND DISCUSSION

Performance and egg quality

Feed intake and rate of egg production of hens were not significantly different across the treatments during the whole experiment (Table 3). Cholesterol content in the egg yolk was not significantly different across the treatments, but numerically increased as increased cheese byproduct. While, Adams et al. (1989) reported that diets containing n-3 PUFA yield a decrease in yolk cholesterol amounts. As shown in Table 2, dietary n-3 PUFA such as C18:3 n-3 was decreased with increasing supplemental level of cheese byproduct. Therefore, the present results could imply that cholesterol level in egg yolk was less affected by CLA compared with n-3 PUFA.

Egg weight, Haugh's unit, eggshell thickness, color, and strength were not significantly different across the treatments. Unlike to the present result, reduced feed intake has been investigated with CLA supplementation in other research with poultry (Ahn et al., 1999; Szymczyk and Pisulewski, 2003; Shang et al., 2004), and egg production rate was decreased with CLA supplementation (Jones et al., 2000). Indeed, other investigation reported that egg weight decreased linearly with increasing dietary CLA (Shnag et al., 2004). Possibly, this is because a cheese flavor could beneficially affect feed intake although dietary CLA has adverse effect on it. Therefore, the present results suggest that supplementation of cheese byproduct including CLA had no adverse effects on layer performance and egg quality.

Fatty acid composition in egg yolk lipids

The amount of C16:0 in egg yolk was not significantly different across the treatments, but that of C18:0 decreased as increased cheese byproduct (Table 4, $p<0.01$). This result indicates that the energy conversion metabolism of fatty acids was not significantly affected by fatty acid supplementation. Monounsaturated fatty acid (MUFA) such as C16:1 and C18:1 maintained similar amount across the treatments. Unlike to the present results, most of researches reported that dietary CLA supplementation decrease the level of MUFA but increased that of SFA in egg yolk (Ahn

Table 4. Fatty acid composition in egg yolk (g/100 g egg yolk lipid)

Fatty acid	Cheese byproduct					SEM	Significance
	0%	1%	3%	5%	10%		
C16:0	26.45	26.06	27.05	26.12	26.03	1.10	NS
C16:1	2.80	2.73	2.99	2.92	2.95	0.51	NS
C18:0	11.16^{a}	11.30^{a}	10.37^{ab}	10.42^{ab}	9.61^{b}	1.10	**
C18:1 n-9	43.59	43.27	42.83	43.54	44.02	2.72	NS
CLA c9t11	ND	0.05^{cd}	0.10^{bc}	0.12^{b}	0.21^{a}	0.07	***
CLA t10c12	ND	ND	ND	ND	ND	ND	-
Total CLA	ND	0.08^{b}	0.12^{b}	0.14^{b}	0.24^{a}	0.08	***
C18:2 n-6	9.51	9.24	9.16	9.03	8.38	1.89	NS
C18:3 n-3	0.14	0.14	0.15	0.13	0.13	0.04	NS
C20:4 n-6	1.78	1.85	1.84	1.73	1.70	0.25	NS
SFA	37.61^{a}	37.36^{a}	37.42^{a}	36.54^{ab}	35.64^{b}	1.47	**
MUFA	46.39	46.00	45.82	46.46	46.97	2.57	NS
PUFA	11.43	11.22	11.14	10.89	10.20	1.97	NS
n-6 PUFA	11.29	11.08	11.00	10.76	10.08	1.94	NS
n-3 PUFA	0.14	0.14	0.15	0.13	0.13	0.04	NS

ND; Not detected, NS; Not significant.
** $p<0.01$ and *** $p<0.001$.
SFA; Saturated fatty acid such as C16:0 and C18:0.
MUFA; Monounsaturated fatty acid such as C16:1 and C18:1.
PUFA; Polyunsaturated fatty acid such as C18:2, C18:3 and C20:4.
n-6 PUFA; C18:2 and C20:4. n-3 PUFA; C18:3.

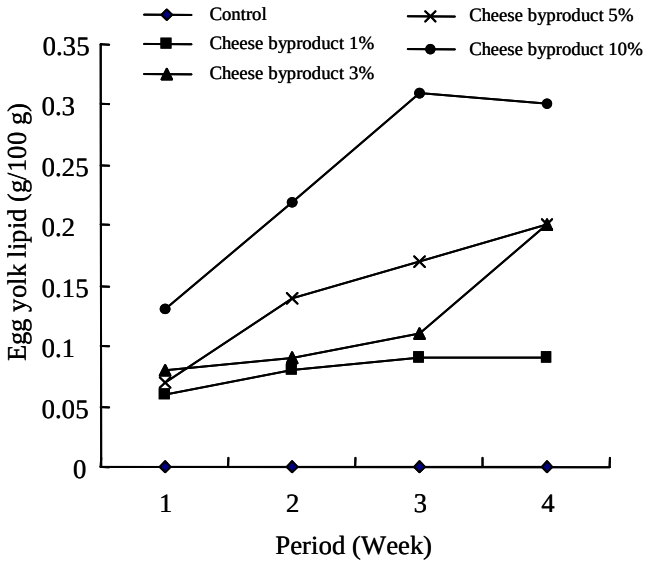

Figure 1. The changes of total CLA in egg yolk lipid during the experiment.

et al., 1999; Chamruspollert and Sell, 1999; Yang et al., 2002; Hwangbo, 2005). Chamrupollert and Sell (1999) suggested that CLA inhibited delta-9 desaturase (stearoyl-CoA desaturase, SCD), which catalyzed the addition of a double bond at the ninth position of C18:0, hence led to an increase in C18:0 and a decrease in C18:1 n-9. It was reported that since the cis-9, trans-11 CLA had no effect, trans-10, cis-12 CLA may have inhibited SCD (Park et al., 2000). Similar to CLA, PUFA such as C18:2 n-6 and C18:3 n-3 have also been reported to decrease SCD activity in liver or adipocytes (Ntambi, 1999). As shown in Table 2, trans-10, cis-12 CLA was not detected across all treatments and C18:2 n-6 and C18:3 n-3 was decreased by increased supplementation level of cheese byproduct. Therefore, the reason of decreased SFA and no change in MUFA level in egg yolk could be explained by the correlation to the dietary fatty acid profile.

As shown in Table 4, it was not even detected when laying hens were fed a normal diet without CLA in egg yolk lipids, and this agrees with other results (Raes et al., 2002; Yang et al., 2002). Thus, the present observation indicated that dietary CLA sources have to be fed to increase CLA amount in egg yolk of hens. It was found that the amount to total CLA in egg yolk lipids reached their maximum after 3-week period (Figure 1). Similar to the present result, Jones et al. (2000) observed that incorporation of CLA into egg yolk lipid was highest on day 24 and 36. While, other studies reported that the period of seems to be sufficient to obtain maximum incorporation of CLA into egg yolk lipid (Chamruspollert and Sell, 1999; Hwangbo, 2005). Total CLA and cis-9, trans-11 CLA amounts were linearly increased by increased cheese byproduct content in feed ($p<0.001$), while trans-10, cis-12 CLA amount was not significantly different across the treatment. Similarly, many investigations have shown that, with increasing dietary CLA, the CLA concentration of egg yolk lipids increased linearly (Ahn et al., 1999; Chamruspollert and Sell, 1999; Cherian et al., 2002). Raes et al. (2002) reported that deposition rate of the cis-9, trans-11 CLA in yolk lipids was higher than the trans-10, cis-12 CLA. This could be associated with the observation that the cis-9, trans-11 CLA is incorporated preferentially relative to the trans-10, ci-12

CLA, despite being fed in approximately the same proportion in the diet (Jones et al., 2000). In addition, Chamruspollert and Sell (1999) found that the cis-9, trans-11 CLA accounted for 50 to 65% of the total CLA in yolk lipid. On the other hand, the present results showed that cis-9, trans-11 CLA accounted for 80% on average of the total CLA.

Total saturated fatty acids (SFA) amount in the egg yolk was decreased as the level of cheese byproduct including CLA was increased ($p<0.01$). However, the amount of monounsaturated fatty acids (MUFA), n-3 polyunsaturated fatty acids (PUFA), n-6 PUFA, and total PUFAs in the egg yolk were not significantly affected by the dietary treatments. Therefore, although CLA has typical negative effects which increase SFA and decrease MUFA, the cheese byproduct as a dietary CLA source showing a desirable possibility to accumulate healthy fatty acids such as CLA, MUFA and PUFAs.

CONCLUSION

The present results suggest that supplementation of cheese byproduct including CLA had not adverse effects on layer performance and egg quality. In addition, beneficial effects were observed that supplementation of cheese byproduct increased the amount of CLA in egg yolk, and that of unsaturated fatty acids in the egg yolk was not negatively influenced by dietary cheese byproduct including CLA. Therefore, it could be concluded that cheese byproduct beneficially improved the fatty acid composition in the egg yolk without adverse effect on egg quality concerning human health.

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