

Effects of Conjugated Linoleic Acid on Production Performance, Egg Quality, Yolk Hardness, and Yolk Fatty Acid Composition in Laying Hens at the Onset of Lay: Postprint

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Abstract

This experiment aimed to investigate the effects of dietary conjugated linoleic acid (CLA) supplementation on production performance, egg quality, yolk hardness, and yolk fatty acid composition in laying hens at the onset of lay. A single-factor randomized experimental design was adopted, selecting 630 18-week-old Hy-Line Brown laying hens with similar laying rate and body weight, which were randomly divided into 7 groups with 6 replicates per group and 15 hens per replicate. The hens were fed experimental diets supplemented with 0, 1%, 2%, 3%, 4%, 5%, and 6% CLA, respectively, with a 1-week pre-trial period and an 8-week formal trial period. The results showed that: 1) When dietary CLA supplementation level was 2%, there was no significant effect on production performance or egg quality of laying hens ($P > 0.05$). Compared with the control group, dietary supplementation of 2%-4% CLA increased eggshell thickness ($P > 0.05$); when dietary CLA supplementation level was 3%, the laying rate was significantly decreased ($P < 0.05$) and yolk hardness was significantly increased ($P < 0.05$); when dietary CLA supplementation level was 4%, average daily feed intake and abdominal fat percentage were significantly decreased ($P < 0.05$); when dietary CLA supplementation level was 5%, feed-to-egg ratio was significantly increased ($P < 0.05$) and yolk percentage was significantly decreased ($P < 0.05$). 2) The contents of C16:0, C18:0, and saturated fatty acids (SFA) in yolk of the 1% and 2% CLA supplementation groups were significantly lower than those in other groups ($P < 0.05$). Compared with the control group, when dietary CLA supplementation level was 4%, the contents of C16:0, C18:0, and SFA in yolk were significantly increased ($P < 0.05$), while the contents of C16:1, C18:1, monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) were significantly decreased ($P < 0.05$). Yolk CLA content

showed a significant quadratic increase with increasing dietary CLA supplementation level ($P < 0.01$), and the enrichment of c9,t11-CLA was 1.47 times that of t10,c12-CLA. In conclusion, dietary supplementation of 2% CLA could improve eggshell quality, reduce yolk SFA content, and enrich yolk with a certain amount of CLA without affecting production performance and egg quality of laying hens.

Full Text

Effects of Conjugated Linoleic Acid on Performance, Egg Quality, Yolk Firmness and Yolk Fatty Acid Composition of Primiparous Laying Hens

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Abstract

This experiment was conducted to investigate the effects of dietary conjugated linoleic acid (CLA) on performance, egg quality, yolk firmness, and yolk fatty acid composition in primiparous laying hens. Using a single-factor randomized design, 630 healthy 18-week-old Hy-Line Brown laying hens with similar body weight and laying rate were randomly allocated to 7 groups with 6 replicates per group and 15 hens per replicate. The hens were fed experimental diets supplemented with 0, 1%, 2%, 3%, 4%, 5%, or 6% CLA for a 1-week adaptation period followed by an 8-week formal experimental period. The results showed: (1) Dietary CLA supplementation at \$ 2% had no significant effects on hen performance or egg quality ($P > 0.05$). Compared with the control group, diets with 2–4% CLA increased eggshell thickness ($P > 0.05$); CLA supplementation at \$ 3% significantly reduced laying rate ($P < 0.05$) and significantly increased yolk firmness ($P < 0.05$); CLA at \$ 4% significantly decreased average daily feed intake and abdominal fat percentage ($P < 0.05$); and CLA at \$ 5% significantly increased feed-to-egg ratio ($P < 0.05$) and significantly reduced yolk percentage ($P < 0.05$). (2) The 1% and 2% CLA groups exhibited significantly lower contents of C16:0, C18:0, and saturated fatty acids (SFA) in yolk compared with other groups ($P < 0.05$). Compared with the control, dietary CLA at \$ 4% significantly increased C16:0, C18:0, and SFA contents ($P < 0.05$) while significantly decreasing C16:1, C18:1, monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) ($P < 0.05$). Yolk CLA

content increased quadratically with dietary CLA level ($P < 0.01$), with c9,t11-CLA enrichment being 1.47 times that of t10,c12-CLA. In conclusion, dietary supplementation with 2% CLA can improve eggshell quality, reduce yolk SFA content, and enrich yolk with a certain amount of CLA without adversely affecting hen performance or egg quality.

Keywords: conjugated linoleic acid; primiparous laying hens; performance; yolk firmness; yolk fatty acid composition

Conjugated linoleic acid (CLA) refers to a group of positional and geometric isomers of linoleic acid (LA). While LA contains two double bonds at positions 9 and 12 in the cis configuration, CLA isomers have double bonds at positions 8,10; 9,11; 10,12; or 11,13 with various cis/trans configurations. Naturally occurring CLA is predominantly found as c9,t11-CLA and t10,c12-CLA. First discovered in grilled beef, CLA is commonly present in meat and milk products from ruminants as an intermediate product of ruminal microbial hydrogenation of LA to stearic acid. As a functional fatty acid, CLA exhibits various physiological functions including anti-mutagenic effects, cancer inhibition, body fat reduction, anti-type II diabetes properties, atherosclerosis prevention, anti-hypertension, antioxidant activity, immune enhancement, and bone health benefits. Eggs are an excellent source of high-quality animal protein, and yolk can effectively accumulate beneficial fatty acids. Feeding laying hens CLA-enriched diets can efficiently deposit CLA into egg yolk, producing CLA-enriched eggs that help balance human diets, improve health, and increase egg value. Although Ahn et al. pioneered research on CLA supplementation in laying hen diets and subsequent studies have been conducted, limited research has examined the efficacy and safety of CLA in primiparous laying hens. This study investigated the effects of dietary CLA supplementation at 1-6% on performance, egg quality, eggshell quality, yolk firmness, and yolk fatty acid composition in primiparous laying hens to provide a scientific basis for CLA application in their diets.

1. Materials and Methods

1.1 Experimental Material CLA was purchased from Qingdao Aohai Biological Co., Ltd. with a purity of 81.4%, containing 38.7% c9,t11-CLA, 38.6% t10,c12-CLA, and 4.1% other CLA isomers (data from the free fatty acid CLA analysis report of Qingdao Aohai Biological Co., Ltd.).

1.2 Experimental Design Six hundred thirty healthy 18-week-old Hy-Line Brown laying hens with similar laying rate and body weight were randomly divided into 7 groups with 6 replicates per group and 15 hens per replicate. No significant differences in initial body weight existed among groups ($P > 0.05$). The control group received corn oil, while treatment groups had CLA oil isocalorically replace corn oil at 1.23%, 2.46%, 3.69%, 4.91%, 6.14%, and 7.37% to achieve actual CLA supplementation levels of 1%, 2%, 3%, 4%, 5%, and 6%,

respectively.

1.3 Husbandry Management A corn-soybean meal basal diet was formulated according to NRC (2004), Chinese Feeding Standard of Chickens (NY/T 33–2004), and the 2015 Hy-Line Brown Commercial Layer Management Guide. Basal diet composition and nutrient levels are shown in Table 1 . Hens were housed in three-tier battery cages (47 cm × 37 cm × 47 cm) with 3 hens per cage, provided ad libitum access to feed and water, and exposed to natural light supplemented with artificial lighting (16 h/d at 20 lx). Relative humidity was maintained at 50–60% with natural ventilation combined with longitudinal negative pressure ventilation. Manure was removed twice daily, hens were disinfected weekly, and routine vaccination and immunization protocols were followed. The adaptation period lasted 1 week (all groups fed the control diet), followed by an 8-week formal experimental period.

1.4 Measurements and Methods

1.4.1 Performance During the experiment, daily egg production and egg weight were recorded per replicate, feed consumption was calculated weekly, and average daily feed intake, laying rate, average egg weight, and feed-to-egg ratio were determined.

1.4.2 Abdominal Fat Percentage At the end of week 8, one hen per replicate with body weight close to the replicate average was selected, weighed, slaughtered by jugular venesection, and abdominal fat was excised and weighed to calculate abdominal fat percentage.

1.4.3 Egg Quality At the end of week 8, three eggs per replicate were randomly collected for egg quality analysis. A SONOVA Egg Analyzer™ (Orka Technology Ltd.) was used to measure albumen height, Haugh unit, and yolk color. Eggshell strength was measured using an Egg Force Reader (Orka Technology Ltd.), and eggshell thickness was determined with an Egg Index Reader (Fujihira Industry Co., Ltd.). Egg weight was measured with an electronic balance, yolk was separated and weighed to calculate yolk percentage, then mixed and stored at -20°C, vacuum freeze-dried (72 h), weighed to calculate yolk moisture content, ground to pass through a 40-mesh sieve, and stored at 4°C.

1.4.4 Yolk Firmness At the end of week 8, six eggs per replicate were randomly collected and divided into two groups. After storage at 4°C for 7 and 14 days, respectively, eggs were boiled and yolks were separated. Yolk firmness was measured using a TMS-PRO food texture analyzer (Food Technology Corporation, USA).

1.4.5 Yolk Fatty Acid Content Yolk freeze-dried powder (90 ± 10 mg) was placed in a 15 mL screw-cap tube, followed by sequential addition of 1 mL n-hexane, 1 mL internal standard solution (1 mg/mL methyl undecanoate in n-hexane), and 4 mL methanol:acetyl chloride mixture (10:1, v/v). After mixing, samples were methylated in an 80°C water bath for 3 h, cooled to room temperature, mixed with 5 mL 7% potassium carbonate solution, vortexed, centrifuged at 4,000 rpm for 10 min, and 1 mL of the upper organic phase was collected for analysis.

A GC-450 gas chromatograph (Techcomp Scientific Instrument Co., Ltd.) equipped with an Agilent HP-88 column (100 m \times 0.25 mm \times 0.20 μ m) was used. Injector temperature was 260°C, detector temperature 270°C, helium as carrier gas at a split ratio of 1:50. The temperature program started at 100°C for 5 min, then increased at 5°C/min to 240°C and held for 30 min. Injection volume was 1.0 μ L with n-hexane as wash solution (3 washes before and after injection).

1.5 Statistical Analysis Data were analyzed using SPSS 19.0 by one-way ANOVA, with Duncan's multiple range test for post-hoc comparisons. Significance was declared at $P < 0.05$, and results are expressed as "mean \pm standard deviation."

2. Results

2.1 Effects of Dietary CLA on Performance of Primiparous Laying Hens As shown in Table 2, dietary CLA supplementation significantly affected average daily feed intake, laying rate, feed-to-egg ratio, average egg weight, and abdominal fat percentage ($P < 0.05$). With increasing CLA levels, average daily feed intake ($y = -1.5196x + 95.456$, $P < 0.01$, $R^2 = 0.965$), laying rate ($y = -0.0279x + 0.7564$, $P < 0.01$, $R^2 = 0.955$), and abdominal fat percentage decreased linearly ($y = -0.2293x + 2.875$, $P < 0.01$, $R^2 = 0.934$), while feed-to-egg ratio increased linearly ($y = 0.0607x + 2.2407$, $P < 0.01$, $R^2 = 0.717$). Based on the regression equation between average egg weight and dietary CLA level ($y = -0.1412x^2 + 0.3921x + 55.682$, $P = 0.017$, $R^2 = 0.840$), the maximum average egg weight (55.95 g) occurred at 1.38% CLA supplementation. Compared with the control, CLA at 2% had no significant effects on performance ($P > 0.05$); CLA at 3% significantly reduced laying rate ($P < 0.05$); CLA at 4% significantly decreased average daily feed intake and abdominal fat percentage ($P < 0.05$); and CLA at 5% significantly increased feed-to-egg ratio ($P < 0.05$) and significantly reduced average egg weight ($P < 0.05$). Dietary CLA had no significant effect on body weight gain ($P > 0.05$).

These results indicate that dietary CLA affects laying hen performance in a dose-dependent manner, with 1% and 2% CLA having no significant effects, while levels 3% influence laying rate, feed intake, and feed-to-egg ratio, and reduce abdominal fat percentage.

2.2 Effects of Dietary CLA on Egg Quality of Primiparous Laying Hens

As shown in Table 3, eggshell thickness in the 2%, 3%, and 4% CLA groups was significantly greater than in the 1% and 5% CLA groups ($P < 0.05$), with the 4% CLA group being significantly thicker than the control ($P < 0.05$). Based on the regression equation between eggshell thickness and dietary CLA level ($y = -0.0019x^2 + 0.0166x + 0.4008$, $P < 0.01$, $R^2 = 0.456$), maximum eggshell thickness (0.4371 mm) occurred at 4.37% CLA. Eggshell strength in the 2%, 3%, and 4% CLA groups showed an increasing trend compared with other groups, though not significant ($P > 0.05$). Yolk percentage tended to decrease with CLA $y = -0.0019x^2 + 0.0166x + 23.491$, $P = 0.069$, $R^2 = 0.847$), maximum yolk percentage (23.50%) occurred at 0.25% CLA supplementation. Dietary CLA had no significant effects on albumen height, yolk color, Haugh unit, or yolk moisture content ($P > 0.05$). These results suggest that dietary CLA at 0.25–4.37% can improve eggshell quality and affect yolk percentage without influencing other egg quality parameters.

2.3 Effects of Dietary CLA on Yolk Firmness of Primiparous Laying Hens

As shown in Table 3, yolk firmness increased linearly with dietary CLA level (7 d: $y = 0.2775x + 2.2918$, $P < 0.01$, $R^2 = 0.942$; 14 d: $y = 0.2514x + 2.5243$, $P < 0.01$, $R^2 = 0.796$). After 7 days of storage at 4°C, yolk firmness in the 3% and 4% CLA groups was significantly higher than the control ($P < 0.05$); the 5% CLA group was significantly higher than the control, 1%, and 2% CLA groups ($P < 0.05$); and the 6% CLA group was significantly higher than all other groups ($P < 0.05$). After 14 days of storage, the 3%, 4%, and 5% CLA groups showed significantly higher yolk firmness than the control, 1%, and 2% CLA groups ($P < 0.05$), while the 6% CLA group was significantly higher than all others ($P < 0.05$). Yolk firmness tended to increase with storage duration. These results indicate that yolk firmness increases with both dietary CLA level and storage time, with CLA 2% having no significant effect and CLA 3% significantly increasing yolk firmness.

2.4 Effects of Dietary CLA on Yolk Fatty Acid Composition of Primiparous Laying Hens

As shown in Table 4, increasing dietary CLA significantly decreased yolk contents of C16:1, C18:1, C18:2, C20:4, MUFA, PUFA, and non-conjugated linoleic acid PUFA ($P < 0.01$), while significantly increasing C14:0, C20:0, and C21:0 ($P < 0.01$). The 1% and 2% CLA groups had significantly lower C16:0, C18:0, and SFA contents than other groups ($P < 0.05$), and significantly lower C17:0 than the 3–6% CLA groups ($P < 0.05$), with a decreasing trend compared with the control. Based on regression equations (C16:0: $y = 0.7968x^2 - 1.9332x + 59.151$, $P < 0.01$, $R^2 = 0.634$; C18:0: $y = 0.6407x^2 - 1.8164x + 15.013$, $P < 0.01$, $R^2 = 0.903$; SFA: $y = 1.9644x^2 - 4.7068x + 77.42$, $P < 0.01$, $R^2 = 0.902$), minimum contents of C16:0 (57.98 mg/g), C18:0 (13.73 mg/g), and SFA (74.60 mg/g) occurred at 1.21%, 1.42%, and 1.20% CLA, respectively. The MUFA regression equation ($y = 2.1964x^2 - 16.938x + 59.381$, $P < 0.01$, $R^2 = 0.873$) indicated minimum MUFA content (26.73 mg/g) at 3.86%

CLA.

Yolk contents of c9,t11-CLA ($y = 0.4344x^2 - 0.3125x + 0.5831$, $P < 0.01$, $R^2 = 0.994$), t10,c12-CLA ($y = 0.2925x^2 - 0.1875x + 0.4014$, $P < 0.01$, $R^2 = 0.974$), and total CLA ($y = 0.7274x^2 - 0.5029x + 0.9855$, $P < 0.01$, $R^2 = 0.990$) increased quadratically with dietary CLA level, with c9,t11-CLA enrichment exceeding that of t10,c12-CLA.

As shown in Table 5, dietary CLA significantly decreased MUFA, PUFA, and non-CLA PUFA contents per egg ($P < 0.01$) while c9,t11-CLA ($y = 2.2254x^2 - 0.0082x + 3.2493$, $P < 0.01$, $R^2 = 0.995$), t10,c12-CLA ($y = 1.4768x^2 + 0.2639x + 2.1714$, $P < 0.01$, $R^2 = 0.972$), and total CLA ($y = 3.7025x^2 + 0.2539x + 5.4257$, $P < 0.01$, $R^2 = 0.990$) increased quadratically ($P < 0.01$). The 1% and 2% CLA groups had significantly lower SFA content than other groups ($P < 0.05$). The regression equation ($y = 8.385x^2 - 20.959x + 516.29$, $P < 0.01$, $R^2 = 0.785$) indicated minimum SFA content per egg (508.43 mg) at 1.25% CLA. The 6% CLA group achieved maximum contents of c9,t11-CLA (83.31 mg), t10,c12-CLA (56.92 mg), and total CLA (140.24 mg) per egg. These results suggest that dietary CLA at 1-2% can reduce yolk SFA content while enriching yolk with CLA.

3. Discussion

3.1 Effects of Dietary CLA on Performance of Primiparous Laying Hens Eggs are nutrient-dense foods that provide high-quality protein and lipids essential for human diets. CLA possesses multiple physiological functions, and combining CLA with egg production to efficiently enrich eggs with CLA without negative effects on hens offers significant potential to enhance egg value, balance human diets, and improve human health.

This study demonstrated that dietary CLA at 1-2% had no significant effects on average daily feed intake, laying rate, feed-to-egg ratio, average egg weight, yolk percentage, or abdominal fat percentage in primiparous laying hens, though these parameters tended to decrease linearly with increasing CLA levels, consistent with Shang et al. Dietary CLA level, hen breed, and age substantially influence performance parameters. Research has shown that 0.5% and 1.0% CLA improved performance in Hy-Line White hens by increasing laying rate, egg weight, and feed intake, whereas 2.0% CLA had opposite effects. Dietary 5% CLA significantly affected average egg weight and yolk weight in 26-week-old White Leghorns but not feed intake or body weight gain, while the same level significantly reduced feed intake in 62-week-old hens without affecting laying rate, egg weight, yolk weight, or body weight gain. In this study, reduced average egg weight and increased feed-to-egg ratio at CLA 3% likely resulted from decreased feed intake and laying rate. Although CLA had no significant effect on body weight gain, abdominal fat percentage decreased linearly with CLA level, possibly due to CLA reducing growth hormone levels, downregulating peroxisome proliferator-activated receptor γ expression, and inhibiting preadipocyte

differentiation, or by activating PPAR α to induce expression of carnitine palmitoyltransferase, acyl-CoA oxidase, and uncoupling proteins, thereby increasing fatty acid β -oxidation. The t10,c12-CLA isomer appears particularly effective in reducing fat deposition.

3.2 Effects of Dietary CLA on Egg Quality of Primiparous Laying Hens This study found that dietary CLA had no significant effects on albumen height, yolk color, Haugh unit, eggshell strength, or yolk moisture content, consistent with our previous research. The greater eggshell thickness and increased eggshell strength trend in the 2-4% CLA groups may be attributed to CLA increasing serum Ca²⁺, P⁵⁺, and alkaline phosphatase activity, elevating serum estradiol levels, and promoting calcium and phosphorus secretion and deposition in the shell gland, or by regulating growth factors and insulin-like growth factor binding protein-3 levels to influence intestinal calcium absorption.

3.3 Effects of Dietary CLA on Yolk Firmness of Primiparous Laying Hens The softness of boiled egg yolk is an important consumer quality attribute. This study demonstrated that CLA-enriched diets significantly increased cooked yolk firmness, elasticity, and resistance to breakage, with effects becoming more pronounced during storage, consistent with previous findings. Increased yolk firmness may be associated with elevated Na⁺, K⁺, and Mg²⁺ concentrations, reduced Ca²⁺, increased yolk pH, and altered moisture content. CLA may also affect yolk membrane permeability; Shinn et al. found that CLA-enriched yolk vitelline membranes had significantly increased C18:0 and decreased C16:1 and C18:1 contents, suggesting CLA inhibits stearoyl-CoA desaturase-1 (SCD-1) activity, preventing C18:0 to C18:1 conversion and altering membrane permeability, leading to changes in yolk composition and increased firmness. This study confirmed that increasing dietary CLA significantly increased yolk SFA content, decreased MUFA content, and increased yolk firmness, consistent with previous reports. The current understanding is that CLA influences yolk firmness by altering fatty acid composition, moisture content, pH, membrane permeability, and ion concentrations between yolk and albumen.

3.4 Effects of Dietary CLA on Yolk Fatty Acid Composition of Primiparous Laying Hens Dietary CLA at 4% significantly increased yolk SFA (C14:0, C16:0, C17:0, C18:0) and decreased MUFA (C16:1, C18:1) contents, consistent with previous studies. Notably, the 1% and 2% CLA groups showed decreased rather than increased C16:0, C18:0, and SFA contents, differing from other studies possibly because corn oil (lower in C16:0 and higher in C18:2 than soybean oil) was used as the control, altering fatty acid metabolism. Differences from Qi et al. may also relate to using 18-week-old hens with more active fatty acid metabolism, where low CLA doses did not significantly affect SFA content. Aydin et al. reported that combining 0.5% CLA with 10% olive oil tended to reduce yolk C16:0 and SFA contents, suggesting that CLA combined

with unsaturated vegetable oils can mitigate CLA' s effects on yolk fatty acid composition.

The mechanism for increased C16:0 and C18:0 contents involves CLA inhibition of hepatic SCD-1 activity. SCD-1, together with NADPH, cytochrome b5 reductase, and cytochrome b5, introduces a double bond into C16:0 and C18:0 to form C16:1 and C18:1. Studies have confirmed that dietary CLA significantly reduces hepatic SCD-1 activity and mRNA expression in laying hens, resulting in increased SFA and decreased MUFA contents. This study also found that c9,t11-CLA, t10,c12-CLA, and total CLA contents increased significantly with dietary CLA level, with c9,t11-CLA enrichment exceeding t10,c12-CLA by up to 1.47-fold, consistent with previous reports. This difference may be due to greater metabolic utilization or functional consumption of t10,c12-CLA.

Reported CLA enrichment in eggs varies widely, with 5% CLA supplementation achieving 310–365 mg CLA per egg, and even 2% CLA reaching 400 mg per egg in some studies. In contrast, this study found 21.28 mg CLA per egg at 2% supplementation and 89.97 mg per egg at 5% CLA, possibly due to differences in hen breed, age, or rearing environment. The differential enrichment of CLA isomers, with c9,t11-CLA exceeding t10,c12-CLA, is consistent with literature reports. Shang et al. reported that CLA \$ 5% did not significantly affect yolk CLA content in dwarf brown hens fed soybean oil + CLA, suggesting a limit to CLA enrichment, which differs from our results in Hy-Line Brown hens fed corn oil + CLA. Our quadratic relationship indicates that yolk CLA content continued increasing up to 6% supplementation without reaching a plateau, likely due to breed differences and dietary oil composition.

4. Conclusions

1. Dietary CLA affects laying hen performance in a dose-dependent manner: 1% and 2% CLA have no significant effects, while levels \$ 3% reduce laying rate, feed intake, and feed-to-egg ratio, and decrease abdominal fat percentage.
2. Dietary CLA at 0.25–4.37% improves eggshell quality and affects yolk percentage without influencing other egg quality traits.
3. Yolk firmness increases with both dietary CLA level and storage duration; CLA \$ 2% has no significant effect, while CLA \$ 3% significantly increases yolk firmness.
4. Dietary CLA at 1–2% reduces yolk SFA content and enriches yolk with CLA.

Table 1 Composition and nutrient levels of the basal diet (air-dry basis)

Items	Content
Ingredients	
Corn	61.50
Soybean meal	23.00
Cottonseed protein	3.00
Wheat bran	2.00
Corn oil	1.50
Limestone	8.00
Calcium hydrogen phosphate	0.60
Sodium chloride	0.30
DL-Methionine	0.15
Premix ¹	0.30
Ethoxyquin	0.02
Total	100.00
Nutrient levels²	
Metabolizable energy (MJ/kg)	11.31
Crude protein	16.50
Available phosphorus	0.40
Lysine	0.75
Methionine	0.38
Methionine + Cysteine	0.65

¹Premix provided the following per kg of diet: VA 12,500 IU, VD₃ 4,125 IU, VE 15 IU, VK 2 mg, thiamine 1 mg, riboflavin 8.5 mg, calcium pantothenate 11 mg, niacin 32.5 mg, pyridoxine 8 mg, biotin 0.5 mg, folic acid 1.25 mg, VB₁₂ 0.02 mg, Mn 65 mg, I 1 mg, Fe 60 mg, Cu 8 mg, Zn 66 mg, choline 1,000 mg, phytase 300 mg, montmorillonite 1,000 mg, yeast culture 10 g.

²Nutrient levels were calculated values.

Table 2 Effects of dietary CLA on performance of primiparous laying hens

Items	Dietary CLA supplemental level/%						P-value
	0	1	2	3	4	5	
							ANOVA

Items	Dietary CLA supplemental level/%	P-value
Average daily feed in-take (g)	95.06±3.29 ^a 93.41±2.58 ^{ab} 93.02±1.00 ^b 90.21±4.40 ^{abc} 89.77±3.42 ^{bc} 88.33±1.97 ^{cd} 85.38±5.05 ^d	< 0.01
Layingrate	(±5.02 ^a 72.77±5.67 ^{ab} 70.02±4.70 ^{abc} 64.60±9.42 ^{bcd} 65.99±7.90 ^{bcd} 62.95±5.74 ^{cd} 57.97 ^d)	< 0.01
Feed – to – egg ratio	2.23±0.08 ^c 2.29±0.17 ^c 2.42±0.16 ^{abc} 2.49±0.33 ^{abc} 2.31±0.29 ^{bc} 2.58±0.12 ^{ab} 2.64±0.29 ^a	< 0.01
Average egg weight (g)	55.78±1.63 ^a 56.03±1.53 ^a 55.12±1.29 ^a 56.23±1.13 ^a 55.31±0.35 ^a 53.60±1.4 ^a	< 0.01
Initial body weight (g)	1721.50±60.51 1693.67±32.39 1681.60±37.77 1711.00±35.58 1696.35±23.12	< 0.01
Body weight gain (g)	58.99±63.72 85.72±28.83 92.90±61.04 53.95±59.49 59.37±75.82 47.44±47.03	< 0.01
Abdominal fat rate	(±0.56 ^{ab} 2.84±0.49 ^a 2.44±0.42 ^{bc} 2.25±0.30 ^{bc} 1.84±0.68 ^{cd} 1.82±0.22 ^{cd} 1.43±0.71 ^d)	< 0.01

In the same row, values with different small letter superscripts mean significant difference (P < 0.05), while with the same or no letter superscripts mean no significant difference (P > 0.05). The same as below.

Table 3 Effects of dietary CLA on egg quality and yolk firmness of primiparous laying hens

Items	Dietary CLA supplemental level/%	P-value							
	0	1	2	3	4	5	6	ANOVA	Mean
Eggshell thickness (mm)	0.42±0.03 ^c 0.44±0.02 ^{ab} 0.44±0.02 ^b 0.45±0.02 ^a 0.42±0.03 ^c 0.43±0.02 ^{abc} 0.42±0.03 ^{bc}	< 0.01							
Eggshell strength (N/mm ²)	33.48±7.06 33.21±6.00 34.30±6.74 34.96±6.24 35.99±5.30 33.48±7.06	< 0.01							
Albumen height (mm)	6.79±0.60 6.15±0.70 6.55±0.92 6.04±0.53 6.11±0.60 6.21±0.48 6.11±0.60	< 0.01							
Yolk color	4.83±0.69 4.89±0.34 4.67±0.56 4.83±0.46 4.83±0.72 4.67±0.30 4.83±0.72 0.19	< 0.01							
Haugh unit	82.31±2.20 77.28±6.68 76.92±4.14 80.43±7.07 77.52±4.76 79.51±4.35 79.72±3.05 0.3	< 0.01							
Yolk percentage	(±0.97 ^{ab} 23.87±0.94 ^a 23.69±0.98 ^a 22.60±0.55 ^{bc} 22.49±0.51 ^{bcd} 21.61±0.77 ^{cd} 21.5	< 0.01							
Yolk moisture content	(±0.53 48.70±0.23 48.76±0.33 48.87±0.17 48.72±0.34 48.82±0.31 49.19±0.3	< 0.01							
Yolk firmness (N)(7d)	2.40±0.27 ^e 2.53±0.32 ^{de} 2.73±0.28 ^c 2.90±0.33 ^{cde} 3.08±0.50 ^{bc} 3.19±0.52 ^{bc}	< 0.01							
Yolk firmness (N)(14d)	2.66±0.33 ^c 2.73±0.26 ^c 3.52±0.56 ^b 3.64±0.54 ^b 3.37±0.54 ^b 4.30±0.71 ^d	< 0.01							

Table 4 Effects of dietary CLA on fatty acids contents of egg yolk of primiparous laying hens (DM basis) (mg/g)

Items	Dietary CLA supplemental level/%						P-value		
	0	1	2	3	4	5		6	ANOVA
C14:0	0.95±0.39 ^b	0.99±0.30 ^b	1.11±0.33 ^a	1.03±0.12 ^a	1.35±0.26 ^{ab}	1.35±0.23 ^{ab}	1.43±0.23 ^a	1.43±0.30 ^a	1.44 < 0.01
C16 :	62.76±3.85 ^c	55.83±4.55 ^d	50.42±3.12 ^d	63.21±7.00 ^c	66.88±4.16 ^{bc}	72.14±4.17 ^{ab}	74.73±5.45 ^a	2.21 < 0.01	
C17 :	2.21±0.42 ^a	0.41±0.08 ^{bc}	1.01±0.22 ^b	0.30±0.04 ^c	1.14±0.15 ^b	0.29±0.06 ^c	1.10±0.25 ^b	0.07 < 0.01	
C18 :	0.21±0.04 ^f	0.15±0.03 ^f	0.67±0.13 ^e	0.64±0.24 ^e	1.18±0.27 ^e	0.65±0.19 ^f	1.78±0.13 ^e	0.78 < 0.01	
C18 :	16.62±1.17 ^c	12.10±1.84 ^d	12.19±0.80 ^d	15.74±1.39 ^c	20.55±2.11 ^b	21.67±2.61 ^b	26.38±2.25 ^a	0.54 < 0.01	
C18 :	61.66±4.94 ^a	36.02±9.62 ^b	27.53±1.44 ^c	29.37±3.13 ^{bc}	31.35±7.36 ^{bc}	29.65±5.93 ^{bc}	32.97±6.06 ^{bc}	1.89 < 0.01	
C18 :	64.68±4.83 ^a	46.84±9.03 ^b	39.78±1.88 ^b	40.46±3.25 ^b	40.08±5.85 ^b	28.02±6.67 ^c	29.47±5.01 ^c	1.95 < 0.01	
C18 :	0.21±0.04 ^f	0.15±0.03 ^f	0.67±0.13 ^e	0.64±0.24 ^e	1.18±0.27 ^e	0.65±0.19 ^f	1.78±0.13 ^e	0.78 < 0.01	
C18 :	0.21±0.04 ^f	0.15±0.03 ^f	0.67±0.13 ^e	0.64±0.24 ^e	1.18±0.27 ^e	0.65±0.19 ^f	1.78±0.13 ^e	0.78 < 0.01	
C20 :	0.63±0.16 ^e	1.00±0.19 ^{de}	1.36±0.37 ^{cd}	1.71±0.42 ^{bc}	9.55±0.62 ^b	5.63±0.30 ^b	4.75±0.64 ^b	0.48 < 0.01	
C20 :	0.19±0.04 ^f	0.65±0.19 ^e	1.39±0.35 ^{de}	1.68±0.50 ^d	3.83±0.29 ^c	2.84±0.34 ^c	3.35±0.63 ^c	0.19 < 0.01	
C20 :	3.48±0.32 ^a	2.19±0.26 ^b	1.55±0.25 ^c	1.36±0.11 ^c	1.28±0.26 ^c	1.31±0.32 ^c	1.38±0.24 ^c	0.12 < 0.01	
C20 :	82.05±4.55 ^c	71.23±4.52 ^d	66.67±3.25 ^d	85.52±8.11 ^c	96.60±5.27 ^b	102.46±5.92 ^b	117.33±6.45 ^a	0.01 < 0.01	
C20 :	63.87±5.09 ^a	40.09±5.89 ^b	28.67±1.57 ^c	30.47±3.27 ^c	32.27±7.51 ^c	30.51±6.09 ^c	33.97±6.17 ^b	0.01 < 0.01	
C20 :	68.32±4.81 ^a	50.78±9.50 ^{bc}	44.52±1.96 ^c	48.65±3.66 ^{bc}	51.87±6.63 ^{bc}	44.67±7.21 ^c	56.32±7.30 ^b	0.01 < 0.01	
C20 :	67.96±4.82 ^a	48.95±9.38 ^b	41.33±1.81 ^b	41.98±3.47 ^b	41.73±6.40 ^b	29.50±6.92 ^c	31.15±5.44 ^c	0.01 < 0.01	
C20 :	0.36±0.06 ^g	1.83±0.45 ^f	3.18±0.46 ^e	6.66±0.51 ^d	10.14±1.08 ^c	15.18±0.54 ^b	25.18±2.14		

Table 5 Effects of dietary CLA on fatty acids contents of egg yolk of primiparous laying hens (mg/egg)

the quality characteristics of chicken eggs during refrigerated storage[J]. Poultry Science, 1999, 78(6): 922-928.

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