

## Effects of Honeysuckle Extract on Production Performance, Egg Quality, Lipid Metabolism, and Yolk Cholesterol Content in Laying Hens (Postprint)

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**Date:** 2017-11-08T00:00:00+00:00

### Abstract

This experiment aimed to investigate the effects of dietary supplementation with different levels of honeysuckle extract on production performance, egg quality, lipid metabolism, and yolk cholesterol content in laying hens. A total of 600 healthy Jingfen No. 2 laying hens at 34 weeks of age with similar laying rates were selected and randomly divided into 4 groups with 5 replicates per group and 30 hens per replicate. The control group was fed a basal diet, while the experimental groups were fed experimental diets supplemented with 100, 200, and 300 mg/kg honeysuckle extract in the basal diet, respectively. The experimental period lasted 35 days. The results showed: 1) Compared with the control group, the 300 mg/kg group exhibited significantly increased average egg weight and average daily feed intake ( $P < 0.05$ ). 2) Compared with the control group, all experimental groups showed significantly improved Haugh units ( $P < 0.05$ ). 3) Compared with the control group, all experimental groups had significantly reduced serum total cholesterol and triglyceride content ( $P < 0.05$ ), the 200 and 300 mg/kg groups showed significantly increased serum high-density lipoprotein cholesterol content ( $P < 0.05$ ), and the 200 and 300 mg/kg groups exhibited significantly decreased yolk cholesterol content ( $P < 0.05$ ). In conclusion, dietary supplementation with honeysuckle extract can increase average egg weight, average daily feed intake, and egg Haugh units, improve lipid metabolism in laying hens, and reduce yolk cholesterol content, with the most pronounced effects observed in the 300 mg/kg group.

## Full Text

# Effects of Honeysuckle Extract on Performance, Egg Quality, Lipid Metabolism and Yolk Cholesterol Content of Laying Hens

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## Abstract

This study investigated the effects of dietary supplementation with different levels of honeysuckle extract on production performance, egg quality, lipid metabolism, and yolk cholesterol content in laying hens. Six hundred healthy Jingfen No. 2 laying hens at 34 weeks of age with similar laying rates were randomly allocated into 4 groups with 5 replicates per group and 30 hens per replicate. The control group received a basal diet, while the experimental groups received the basal diet supplemented with 100, 200, or 300 mg/kg honeysuckle extract. The 35-day trial period consisted of a 7-day preliminary period and a 28-day formal experimental period. The results showed: (1) Compared with the control group, the 300 mg/kg group exhibited significantly increased average egg weight and average daily feed intake ( $P < 0.05$ ). (2) The Haugh unit was significantly improved in all experimental groups compared with the control group ( $P < 0.05$ ). (3) Serum total cholesterol and triglyceride contents were significantly reduced in all experimental groups ( $P < 0.05$ ), while serum high-density lipoprotein cholesterol content was significantly increased in the 200 and 300 mg/kg groups ( $P < 0.05$ ). Additionally, yolk cholesterol content was significantly decreased in the 200 and 300 mg/kg groups ( $P < 0.05$ ). These findings indicate that dietary honeysuckle extract supplementation can increase average egg weight, daily feed intake, and Haugh unit, improve lipid metabolism, and reduce yolk cholesterol content, with the 300 mg/kg level showing the most pronounced effects.

**Keywords:** honeysuckle extract; laying hens; performance; egg quality; yolk cholesterol

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## Introduction

Honeysuckle (*Lonicera japonica* Thunb.) is the dried flower bud of plants from the Caprifoliaceae family and represents a traditional Chinese medicinal herb. Its primary chemical constituents include phenolic acids, flavonoids, volatile oils, and triterpenoid saponins. The main active component of honeysuckle extract is chlorogenic acid (CGA), which has been reported to possess antioxidant, antibacterial, and antiviral properties that can enhance immune function. Recent

research indicates that CGA plays a significant role in regulating glucose and lipid metabolism. However, few studies have investigated the effects of honeysuckle extract on yolk cholesterol content and lipid metabolism in laying hens. Therefore, this study utilized 34-week-old Jingfen No. 2 laying hens to evaluate the effects of dietary supplementation with varying levels of honeysuckle extract on production performance, egg quality, lipid metabolism, and yolk cholesterol content, thereby exploring the potential for reducing egg cholesterol levels and providing a scientific basis for the application of honeysuckle extract in egg production.

**Received:** 2017-07-02

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## 1. Materials and Methods

**1.1 Experimental Material** Honeysuckle extract was purchased from Hunan Greenvines Biotechnology Co., Ltd., with an active chlorogenic acid content of 25%.

**1.2 Experimental Design and Management** Six hundred healthy Jingfen No. 2 laying hens at 34 weeks of age with similar body weight and laying performance were randomly divided into 4 groups with 5 replicates per group and 30 hens per replicate. The control group received a basal diet without honeysuckle extract, while the experimental groups received the basal diet supplemented with 100, 200, or 300 mg/kg honeysuckle extract (providing 25, 50, and 75 mg/kg CGA, respectively). The composition and nutrient levels of the basal diet are presented in Table 1 .

Feed was provided twice daily at 06:30 and 14:30. Hens were housed in three-tier step cages with ad libitum access to feed and water. The lighting regimen consisted of 16 hours of light per day, and manure was removed once daily. The experimental period lasted 35 days, including a 7-day preliminary period and a 28-day formal experimental period.

### Table 1 Footnote:

1) Per kilogram of premix contained: VA 4,000,000 IU, VD 600,000 IU, VE 20 g, VK 500 mg, VB 50 mg, VB 3 g, nicotinic acid 1,500 mg, pantothenic acid 70 mg, folic acid 500 mg, choline chloride 20 g, Fe 60 g, Cu 10 g, Mn 50 g, Zn 70 g, Se 25 mg.

2) Nutrient levels were calculated values.

### 1.3 Measurement Indicators 1.3.1 Production Performance

On a replicate basis, daily records were maintained for egg weight, egg number,

feed allocation, residual feed, hen mortality, and broken/soft-shelled eggs. Average egg weight, laying rate, average daily feed intake, broken egg rate, and feed-to-egg ratio were calculated.

### 1.3.2 Egg Quality

On the final day of the experiment, 20 eggs were randomly collected from each group (4 eggs per replicate) to determine physical quality parameters including egg shape index, shell strength, yolk color, shell thickness, and Haugh unit. Measurements were conducted using an egg quality tester (Shenyang Fushiping Industrial Co., Ltd.), shell strength tester (Shenyang Fushiping Industrial Co., Ltd.), and Roche color fan (Roche, USA).

### 1.3.3 Yolk Cholesterol Content

Ten eggs were randomly collected from each group (2 eggs per replicate) for yolk cholesterol analysis. Eggs were boiled, and yolks were ground uniformly. One gram of yolk was extracted with methanol-chloroform solution (1:3), ground until no visible particles remained, mixed for 8 minutes, and filtered into a 10 mL volumetric flask. The solution was brought to volume with methanol-chloroform. Subsequently, 0.02 mL of yolk filtrate, cholesterol standard solution, and methanol-chloroform solution were added to sample, standard, and blank tubes, respectively. Three milliliters of o-phthalaldehyde reagent and 2 mL of concentrated sulfuric acid were added to each tube, mixed, and cooled. A T-6 UV-Vis spectrophotometer (Beijing Puxi) was used to measure absorbance at 560 nm against the blank tube, and yolk cholesterol content was calculated according to the method described by Bai et al. [6].

### 1.3.4 Lipid Metabolism

At the end of the experiment, one hen was randomly selected from each replicate, and blood was collected from the wing vein. Serum was separated by centrifugation at 3,000 r/min for 10 minutes and stored at -20°C. Serum total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) contents were determined using assay kits from Nanjing Jiancheng Bioengineering Institute.

**1.4 Statistical Analysis** Experimental data were analyzed using one-way ANOVA with SPSS 20.0 software. Duncan's multiple range test was used for post-hoc comparisons. Results are expressed as means  $\pm$  standard deviation.

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## 2. Results

**2.1 Effects of Honeysuckle Extract on Production Performance** As shown in Table 2, no significant differences were observed in laying rate, feed-to-egg ratio, or broken egg rate among groups ( $P > 0.05$ ). Average egg weight exhibited a trend of initial decrease followed by increase with increasing honeysuckle extract levels. The 100 and 200 mg/kg groups showed no significant difference in average egg weight compared with the control group ( $P > 0.05$ ),

whereas the 300 mg/kg group demonstrated significantly increased average egg weight ( $P < 0.05$ ). Additionally, the 300 mg/kg group exhibited significantly higher average daily feed intake compared with the control group ( $P < 0.05$ ).

**Table 2 Note:** In the same row, values with the same or no letter superscripts indicate no significant difference ( $P > 0.05$ ), while different letter superscripts indicate significant difference ( $P < 0.05$ ). The same notation applies to subsequent tables.

**2.2 Effects of Honeysuckle Extract on Egg Quality** Table 3 shows that no significant differences were observed in egg shape index, yolk color, shell thickness, or shell strength among all groups ( $P > 0.05$ ). However, the Haugh unit was significantly improved in all experimental groups compared with the control group ( $P < 0.05$ ), with increases of 8.2%, 6.2%, and 9.2% for the 100, 200, and 300 mg/kg groups, respectively.

**2.3 Effects of Honeysuckle Extract on Lipid Metabolism and Yolk Cholesterol Content** As presented in Table 4, serum TG content was significantly reduced in all experimental groups compared with the control group ( $P < 0.05$ ). Serum TC content was also significantly decreased in all experimental groups ( $P < 0.05$ ), with the most pronounced reduction (27.56%) observed in the 300 mg/kg group. Serum HDL-C content increased with increasing honeysuckle extract levels, with the 200 and 300 mg/kg groups showing significantly higher values than the control group ( $P < 0.05$ ). No significant differences were observed in serum LDL-C content among groups ( $P > 0.05$ ). Yolk cholesterol content was significantly reduced in the 200 and 300 mg/kg groups compared with the control group ( $P < 0.05$ ), with decreases of 8.17% and 18.64%, respectively.

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### 3. Discussion

**3.1 Effects of Honeysuckle Extract on Production Performance** The results indicate that dietary supplementation with 300 mg/kg honeysuckle extract significantly increased average daily feed intake and average egg weight in laying hens. The primary active component of honeysuckle extract, chlorogenic acid (CGA), exhibits multiple biological functions including antioxidant, antibacterial, antiviral, and immune-enhancing activities. Zhang [8] reported that dietary supplementation with 0.5%, 1.0%, 1.5%, and 2.0% honeysuckle powder in Roman laying hens increased average daily feed intake by 5.46%, 8.20%, 9.53%, and 9.63%, respectively, and significantly improved laying rate at supplementation levels of 1.0%, 1.5%, and 2.0%. Huang et al. [9] found that dietary supplementation with 0.15% CGA from *eucommia* leaves significantly increased average daily weight gain in broilers aged 7-14 and 28-35 days, while 0.05% and 0.15% CGA reduced feed-to-gain ratio by 21.15% and 19.34% during 28-35 days, respectively. These findings demonstrate that CGA positively influences animal production performance, possibly due to its potent antioxidant

capacity and inhibitory effects on lipid peroxidation. Guan et al. [11] reported that the antioxidant capacity of honeysuckle extract correlates with its CGA content, and its hydroxyl radical scavenging ability surpasses that of vitamin C. Zang et al. [12] found that CGA directly scavenges hydroxyl radicals with a rate constant of  $7.73 \times 10^9$ . Li et al. [13] demonstrated that CGA effectively scavenges superoxide anions, hydroxyl radicals, alkyl peroxy radicals, and DPPH radicals, with superior activity against alkyl peroxy radicals and superoxide anions compared to vitamin C. Furthermore, CGA's antibacterial and antiviral properties may enhance poultry immunity and improve production performance. Studies have shown that CGA can augment T-cell proliferation induced by influenza virus antigens [14] and significantly increase immunoglobulin E (IgE), immunoglobulin G (IgG), and interleukin-4 (IL-4) levels in rats [15]. Ma et al. [16] reported that CGA significantly increased tumor necrosis factor- and interferon- in rat intestinal lamina propria lymphocytes.

**3.2 Effects of Honeysuckle Extract on Egg Quality** The results showed that honeysuckle extract supplementation had no significant effects on egg shape index, yolk color, shell thickness, or shell strength. However, all experimental groups exhibited significantly improved Haugh units, indicating enhanced albumen height and potentially improved protein secretion. Zhang [8] found that dietary supplementation with 0.5% and 1.5% honeysuckle powder modestly increased Haugh units, though the differences were not statistically significant. Egg protein secretion primarily occurs in the magnum portion of the oviduct, where honeysuckle extract may exert its effects. The active component CGA possesses strong antioxidant capacity that may help maintain normal physiological functions of secretory cell membranes, although the exact mechanism remains unclear. Shao et al. [17] suggested that free radicals can modify amino acids, peptides, and proteins, promoting protein degradation, while CGA in honeysuckle extract effectively scavenges these radicals.

**3.3 Effects of Honeysuckle Extract on Lipid Metabolism and Yolk Cholesterol Content** Cholesterol in laying hens originates from two primary pathways: (1) exogenous cholesterol from the diet, which is negligible as layer diets typically contain no cholesterol; and (2) endogenous synthesis, primarily in the liver, where very-low-density lipoprotein and vitellogenin serve as precursors for yolk cholesterol, which is then transported via circulation to the ovary for deposition in the yolk. Cholesterol is eliminated through three main routes: (1) excretion via eggs, accounting for approximately two-thirds of total cholesterol; (2) fecal excretion of neutral sterols produced by the liver and intestine; and (3) fecal elimination of bile acids formed from oxidized cholesterol in the liver [19]. This study found that yolk cholesterol content decreased by 8.17% and 18.64% in the 200 and 300 mg/kg groups, respectively. The mechanisms by which CGA, the primary active component in honeysuckle extract, influences lipid metabolism may include: (1) Modulation of cholesterol synthesis and catabolism through effects on key enzyme activities and gene

expression. Li et al. [20] reported that eucommia leaf CGA extract inhibited hepatic 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase activity more potently than simvastatin. HMG-CoA reductase is the rate-limiting enzyme in cholesterol synthesis. Ong et al. [21] demonstrated that CGA administered at 250 mg/kg to db/db mice improved glucose and lipid metabolism by activating AMP-activated protein kinase (AMPK), thereby affecting the AMPK-HMG-CoA reductase (HMGR) pathway. AMPK phosphorylates HMGR at the Ser-871 residue [22]. Studies investigating the effects of varying concentrations of 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR) on hepatic HMGR activity found that AMPK activation occurred in a dose-dependent manner, concurrently with dose-dependent inactivation of HMGR [23-24]. Therefore, CGA may inhibit cholesterol synthesis by activating AMPK, leading to phosphorylation and inactivation of HMGR. Cholesterol 7 $\alpha$ -hydroxylase (CYP7A1) is the rate-limiting enzyme for cholesterol conversion to bile acids in the liver [25]. Wan et al. [26] reported that CGA supplementation (1 and 10 mg/kg) in high-cholesterol diets upregulated CYP7A1 expression, promoting cholesterol conversion to bile acids. (2) Regulation of cholesterol synthesis through modulation of sterol regulatory element-binding proteins (SREBPs). Murase et al. [27] found that 5  $\mu$ mol/L CGA downregulated mRNA expression of acetyl-CoA carboxylase, fatty acid synthase, and SREBP-1c in Hepa1-6 cells. (3) Enhancement of reverse cholesterol transport and bile acid excretion through activation of peroxisome proliferator-activated receptors (PPARs). Li et al. [28] reported that CGA administration (80 mg/kg body weight) to male golden hamsters fed a high-fat diet significantly increased hepatic PPAR- mRNA and protein expression. Fruchart et al. [29] proposed that PPAR activation influences five genes associated with high-density lipoprotein (HDL), including apolipoprotein A (apo-A), apolipoprotein B (apo-B), lipoprotein lipase (LPL), scavenger receptor class B type I (SR-BI), and ATP-binding cassette transporter A1 (ABCA1), thereby promoting HDL formation, increasing serum HDL content, and facilitating reverse cholesterol transport. The present study demonstrated that honeysuckle extract significantly reduced serum TC and TG contents, with the most pronounced effects observed in the 300 mg/kg group. The 200 and 300 mg/kg groups also increased serum HDL-C content, promoting reverse cholesterol transport.

In summary: (1) Dietary supplementation with 300 mg/kg honeysuckle extract significantly increased average egg weight and daily feed intake without adversely affecting production performance. (2) Honeysuckle extract supplementation significantly improved egg Haugh unit. (3) Honeysuckle extract improved lipid metabolism by increasing serum HDL-C content, promoting reverse cholesterol transport, and reducing yolk cholesterol content.

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